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**Articles and Statements**

# A Study of Antagonistic Relations of Microorganisms to Some Phytopathogenic Bacteria

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**Abstract**

The relation of tubercle (Rhizobium trifoli, Rhizobium arachis, Rhizobium meliloti, Rhizobium phaseoli, Rhizobium lupini, Rhizobium leguminosarum) and rhizosphere microorganisms of legumes to some phytopathogenic bacteria (Pseudomonas tumefaciens, Corynebacterium michiganense, Pectobacterium carotovora, Pectobacterium aroidea, Xanthomonas vesicatoria, Pectobacterium phytophtorum, Xanthomonas campestris, Pseudomonas syringae) has been studied. Tubercle bacteria were found to reveal strong antagonistic properties, whereas rhizosphere microorganisms – medium.

**Keywords:** phytopathogenic bacteria, legume plants, antagonistic, rhizosphere, microflora.

## 1. Введение

В последнее время большое внимание уделяют использованию биологических методов в борьбе по охране растений, как одно из перспективных средств. В связи с этим ведутся обширные исследования по изучению антибиотических свойств многих микроорганизмов для того, чтобы это использовалось против фитопатогенных бактерий (Егоров, 1995).

По данным многих авторов установлено, что фитопатогенные бактерии не могут долго существовать в почве. Это наряду со многими факторами также обусловлено антагонистическим действием микрофлоры почвы (Кретович, 1994, Доросинский, 1970, Определитель бактерий Берджи, 1997).

Целью исследования является установление независимости микроорганизмов нароста и ризосферы по отношению к некоторым фитопатогенным бактериям.

По данным Воронкевича (Elsas, 2007) известно, что фитопатогенные бактерии долго живут в ризосфере растения и их жизнеспособность более активна. Это обусловлено взаимосвязью фитопатогенных бактерий и бактерий ризосферы.

В преобразовании атмосферного азота и увеличении оплодотворения почвы важную роль играют бобовые растения. Бобовые растения (люцерна, горох, люпин, фасоль) кроме высокого содержания белка, содержат также витамины; их широко используют в севообороте многих культур (Желдакова, Мянин, 2006). Вышеуказанные растения

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инфицируются разными бактериальными заболеваниями, поэтому изучение взаимосвязи микроорганизмов ризосферы, нароста и фитопатогенных бактерий важно, как с теоретической, так и с практической точки зрения.

## 2. Материалы и методы

Объектом исследования были взяты бобовые растения – люцерна, горох, люпин, фасоль. В фазе цветения из их нароста были выделены бактерии по методу Израильского, которые были проверены на образование нароста (Котляров, 2008).

Для установления видов бактерий, выделенных из нароста, были изучены их морфологические, культуральные и биохимические свойства. Из ризосферы бобовых растений микроорганизмы выделяли по методу Берёзовой (Гусев, Минеева, 2010; Егоров, 1965).

Для исследования использовали некоторые фитопатогенные бактерии, полученные из микробиологической лаборатории института защиты растений имени Л. Карчавели, которые вызывают разные сельскохозяйственные заболевания: *Pseudomonas tumefaciens* (47) – бактериальный рак винограда, *Corynebacterium michiganense* – бактериальный рак томатов, *Pectobacterium carotovora* (54) и *Pectobacterium aroidea* (137) – слизистость капусты, *Xanthomonas vesicatoria* (25) – бактериальная пятнистость томатов, *Pectobacterium phytophtorum* (997) – чёрная ножка картофеля, *Xanthomonas campestris* (277) – сосудистый бактериоз капусты и *Pseudomonas syringa* – пятнистость листьев и плодов фруктовых деревьев (яблоня-груша), а также некоторые бактериальные штаммы наростов *Rhizobium trifoli* (115), *Rhizobium arachis* (870).

Антагонистические свойства наростов и некоторых микроорганизмов ризосферы мы устанавливали биологическим путём – методом блокировки (Воронькевич, 1974).

Антагонистическое действие бактерий нароста и микроорганизмов ризосферы в отношении фитопатогенных бактерий мы учитывали на третий, пятый, седьмой и десятый дни.

## 3.Результаты и обсуждение

В результате проведённой работы было установлено, что бактерии, выделенные из наростов люцерны, гороха, люпина, фасоли, своими морфологическими, культуральными и биохимическими свойствами принадлежат следующим видам: из люцерны *Rhizobium meliloti* (Dangeard), из фасоли – *Rhizobium phaseoli* (Dangeard), из люпина – *Rhizobium lupini* (Schroeter), а из гороха – *Rhizobium leguminosarum* (Frank). Было изучено их антагонистическое действие в отношении фитопатогенных бактерий *Xanthomonas campestris* (277), *Pectobacterium aroidea* (137), *Pseudomonas syringae*, *Corynebacterium michiganense*, *Pseudomonas tumefaciens* (47), *Xanthomonas vesicatoria* (25), *Pectobacterium carotovora* (54), *Pectobacterium phytophtorum* (997) (см. Таблицу 1).

**Таблица 1.** Антагонистическое действие бактерий нароста на фитопатогенные бактерии

| № | Бактерии нароста | Фитопатогенные бактерии | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Pectobacterium aroidea* (137) | *Corynebacterium michiganense* | *Pectobacterium carotovora* (54) | *Pseudomonas tumefaciens* (47) | *Xanthomonas campestris* (277) | *Xanthomonas vesicatoria* (23) | *Pectobacterium phytophtorum* (997) | *Pseudomonas syringae* |
| 1 | *Rhizobium meliloti* | – | – | – | – | – | – | – | – |
| 2 | *Rhizobium phaseoli* | – | – | – | – | – | – | – | – |
| 3 | *Rhizobium arachis* (870) | – | – | + | – | +++ | + | – | – |
| 4 | *Rhizobium trifoli* (115) | – | – | + | – | – | – | – | – |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | *Rhizobium leguminosarum* | +++ | – | – | – | – | +++ | – | – |
| 6 | *Rhizobium lupini* | – | – | + | – | – | + | – | – |

Бактерии нароста 1) *Rhizobium arachis* (870), *Rhizobium leguminosarum* (Frank) проявляют сильные антагонистические свойства в отношении *Pectobacterium aroidea* (137), *Xanthomonas vesicatoria* (25) ϖ *Xanthomonas campestris* (277), а *Rhizobium trifoli* (115), *Rhizobium lupini* (Schroeter), *Rhizobium arachis* (870) проявляют слабые антагонистические свойства в отношении *Pectobacterium carotovora* (54), *Xanthomonas vesicatoria* (25). Особенно сильные антагонистические свойства проявляются на пятый-седьмой день.

Антагонистические свойства штаммов 4, 7, 10, 12 и 17 бактерий выделенных из ризосферы люцерны, гороха, люпина, фасоли были изучены в отношении вышеуказанных фитопатогенных бактерий (см. Таблицу 2).

**Таблица 2.** Антагонистическое действие бактерий, выделенных из ризосферы бобовых растений на фитопатогенные бактерии

| Номера штаммов выделенных из ризосферы | Фитопатогенные бактерии | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Pectobacterium aroidea* (137) | *Corynebacterium michiganense* | *Pectobacterium carotovora* (54) | *Pseudomonas tumefaciens* (47) | *Xanthomonas vesicatoria* (25) | *Pectobacterium phytophtorum* (997) | *Pseudomonas syringae* |
| 4 | + | – | ++ | – | – | + | – |
| 7 | – | + | – | – | – | – | + |
| 10 | – | – | – | + | – | + | – |
| 12 | + | – | ++ | – | – | – | + |
| 17 | – | – | – | + | + | – | – |

Выявлено, что штамм 4 и 12 проявляют средние антагонистический свойства в отношении фитопатогенных бактерий *Pectobacterium carotovora* (54), а слабые антагонистические свойства в отношении *Pectobacterium aroidea* (137). Остальные штаммы проявляют слабые антагонистические свойства в отношении всех вместе взятых для испытания фитопатогенных бактерий.

В Таблицах 1, 2 антагонистические свойства бактерий ризосферы нароста были рассмотрены согласно величине возникших зон, которая измеряется в мм; «+» показывает слабые антагонистические свойства, «++» показывает средние антагонистические свойства, а «+++» сильные антагонистические свойства; «–» показывает, что антагонистические свойства не проявляются.

Таким образом, было установлено, что из наростов бобовых растений выделены следующие бактерии: *Rhizobium meliloti* (Dangeard), *Rhizobium phaseoli* (Dangeard), *Rhizobium lupini* (Schroeter), *Rhizobium leguminosarum* (Frank). Оказалось, что сильные антагонистические свойства в отношении некоторых фитопатогенных бактерий проявляют бактерии наростов, а средние – некоторые бактерии ризосферы.

**Литература**

Воронькевич, 1974 – *Воронькевич И.В.* Выживаемость фитопатогенных бактерий в природе. Москва: Наука, 1974.

Гусев, Минеева, 2010 – *Гусев М.В., Минеева Л.А. Микробиология*. 9-е изд., стер. М.: Издательский центр «Академия», 2010.

Доросинский, 1970 – *Доросинский Л.М.* Клубеньковые бактерии и нитрагин. ЛенингрАД: Колос, 1970.

Егоров, 1965 – *Егоров Н.С.* Микробы антагонисты и биологические методы определения антибиотической активности. Москва: Высшая школа, 1965.

Егоров, 1995 – *Егоров Н.С.* Руководство к практическим занятиям по микробиологии. Москва: МГУ, 1995.

Желдакова, Мянин, 2006 – *Желдакова Р.А., Мянин В.Е.* Фитопатогенные микроорганизмы. Минск: БГУ, 2006.

Котляров, 2008 – *Котляров В.В. Бактериальные болезни культурных растений*. Краснодар: КубГАУ, 2008.

Кретович, 1994 – *Кретович В.Л.* Биохимия усвоения азота воздуха растениями. Москва: Наука, 1994.

Определитель бактерий Берджи, 1997 – Определитель бактерий Берджи. В 2-х т. (1997). Т. 2: Пер. с англ. / Под ред. Дж. Хоула, Н. Крига, П. Снита, Дж. Стейли, С. Уилльямса. Москва: Мир.

Elsas, 2007 – *Elsas Jan D., Jansson Janet K., Trevors Jack T.* Modern soil microbiology. CRC Press/Taylor & Francis, 2007.

**References**

Dorosinskii, 1970 – *Dorosinskii, L.M.* (1970). Kluben'kovye bakterii i nitragin [Nodule bacteria and nitragin]. LeningrAD: Kolos. [in Russian]

Egorov, 1965 – *Egorov, N.S.* (1965). Mikroby antagonisty i biologicheskie metody opredeleniya antibioticheskoi aktivnosti [Microbes antagonists and biological methods for determining antibiotic activity]. Moskva: Vysshaya shkola. [in Russian]

Egorov, 1995 – *Egorov, N.S.* (1995). Rukovodstvo k prakticheskim zanyatiyam po mikrobiologii [Guide to practical exercises in microbiology]. Moskva: MGU. [in Russian]

Elsas, 2007 – *Elsas, Jan D., Jansson, Janet K., Trevors, Jack T.* (2007). Modern soil microbiology. CRC Press/Taylor & Francis.

Gusev, Mineeva, 2010 – *Gusev, M.V., Mineeva, L.A.* (2010). Mikrobiologiya. 9-e izd. [Microbiology. 9th ed.], ster. M.: Izdatel'skii tsentr «Akademiya». [in Russian]

Kotlyarov, 2008 – *Kotlyarov, V.V.* (2008). Bakterial'nye bolezni kul'turnykh rastenii [Bacterial diseases of cultivated plants]. Krasnodar: KubGAU. [in Russian]

Kretovich, 1994 – *Kretovich, V.L.* (1994). Biokhimiya usvoeniya azota vozdukha rasteniyami [Biochemistry of the assimilation of air nitrogen by plants]. Moskva: Nauka. [in Russian]

Opredelitel' bakterii Berdzhi, 1997 – Opredelitel' bakterii Berdzhi [Bergey's determinant of bacteria]. V 2-kh t. (1997). T. 2: Per. s angl. / Pod red. Dzh. Khoula, N. Kriga, P. Snita, Dzh. Steili, S. Uill'yamsa. Moskva: Mir. [in Russian]

Voron'kevich, 1974 – *Voron'kevich, I.V.* (1974). Vyzhivaemost' fitopatogennykh bakterii v prirode [Survival of phytopathogenic bacteria in nature]. Moskva: Nauka. [in Russian]

Zheldakova, Myanin, 2006 – *Zheldakova, R.A., Myanin, V.E.* (2006). Fitopatogennye mikroorganizmy [Phytopathogenic microorganisms]. Minsk: BGU. [in Russian]

## Изучение антагонистического отношения микроорганизмов к некоторым фитопатогенным бактериям

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**Аннотация.** Было изучено отношение клубеньковых (*Rhizobium trifoli, Rhizobium arachis, Rhizobium meliloti, Rhizobium phaseoli, Rhizobium lupini, Rhizobium leguminosarum*) и ризосферных микроорганизмов бобовых растений к некоторым

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фитопатогенным бактериям (*Pseudomonas tumefaciens, Corynebacterium michiganense, Pectobacterium carotovora, Pectobacterium aroidea, Xanthomonas vesicatoria, Pectobacterium phytophtorum, Xanthomonas campestris, Pseudomonas syringae*). Установлено, что клубеньковые бактерии проявляют сильные антагонистические свойства, а ризосферные микроорганизмы – средние.

**Ключевые слова:** фитопатогенные бактерии, бобовые растения, антагонистический, ризосфера, микрофлора.

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# Evaluation of the Antiplasmodial Activity of Diethyl Ether Leaf Extract of Eucalyptus Camaldulensis in Experimental Mice

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**Abstract**

The main aim of this research was to study the *in vivo* antiplasmodial activity of diethyl ether fraction of *Eucalyptus camaldulensis* leaf on malaria infection in experimental albino mice. The dried leaves were pounded into its powdered form with the aid of mortar and pestle. The plant leaf was extracted with Diethyl ether, yielded 4.03 g extract and the extract was screened for antiplasmodial activity. Mice infected with *Plasmodium berghei* were administered intraperitoneally doses ranging from 100-300 mg/kg body weight/day of the extract for 10 consecutive days. The positive control group was treated with 25 mg/kg of chloroquine. The diethyl ether extracts of *Eucalyptus camaldulensis* leaf shows suppressive activities on *Plasmodium berghei* in the animals treated. The group treated with standard drug (chloroquine) and the normal control group (uninfected untreated) survived beyond the experimental period, although the clearance time was faster for the standard drug. Changes in behavior and bodyweight were observed, this could be as a result of loss of appetite in the treated animal. Phytochemical screening revealed the presence of 6 phytochemicals including cardiac glycosides. The packed cell volume of the groups treated with diethyl ether extract of *Eucalyptus camaldulensis* decreased significantly as compared with both the positive and negative control groups while there is a slight increase in the normal control groups. From the result obtained, we can conclude that the diethyl ether leaf extract of *Eucalyptus camaldulensis* at 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of mice reduced parasitemia level. So diethyl ether fraction of *Eucalyptus camaldulensis* was effective in the therapeutic management of malaria parasite.

**Keywords:** Plasmodium berghei, Eucalyptus camaldulensis, diethyl ether, packed cell volume, parasitemial load, phytochemicals.

## 1. Introduction

*Plasmodium falciparum*, the pathogen most widespread human malaria, is becoming increasingly resistant to antimalarial drugs deal. This requires extra effort and continuous search for new drugs, especially with new modes of action (Muregi et al., 2003). In Sub-Saharan Africa, the proportion of patients utilizing antimalarial treatments outside the official circuit varies from 12 to 80 % (Bloland et al., 2000). Ethno medical and ethnobotanical studies now recognized to be the most viable methods of identifying new medicinal plants (Igoli et al., 2005). The use of medicinal plants plays an important role in daily health care in most rural area. Among some

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ethnic groups of Southern Cameroon like Pygmées-Baka, local medicine remains more popular than western medicine (Titanji et al., 2008). Traditional plants may supplement and/or even replace effective drug manufacturers, often inaccessible, for the treatment of malaria. Herbal medicine remains one of the common forms of therapy available for people worldwide.

*Eucalyptus camaldulensis* extract has many properties such as anti-oxidant, anti-blood proliferation, anti-cancer, anti-inflammatory, and anti-mold, painkiller anti-parasitic, anti-microbial, repellent and anti-virus properties.

Malaria life cycle involves two hosts and varies according to the type of plasmodium parasite that is causing the infection. There are slight variations in the life cycle of the plasmodium.

Therefore, aim of this research was to evaluate the *in vivo* antiplasmodial activity of diethyl ether extract of *Eucalyptus camaldulensis* leaf on malaria infection in experimental albino mice.

## 2. Materials and methods

### Equipments

Conical flask, Bauchner funnel-flask, Filter paper, Cotton wool, Vacuum pump, Beakers, Evaporating dish, Mortar and pestle.

### Reagents/Chemicals

Diethyl ether, Sulfuric acid, Chloroform, Benedict reagents, Hydrochloric acid, Meyer's reagent, Picric acid solution, Sodium hydroxide, Dragendorff's reagent, Ferric chloride reagent, Acetic anhydride, Water, Ammonia solution, Molisch reagent.

### Materials for Infection of Experimental Mice

Methanol, Cotton wool, Normal saline, Razor blade, Butterfly needle, 2 ml syringe, Beaker, Slides, Giemsa stain, Microscope, Hematocrit centrifuge.

### Preparation of Plant Material

The plant was collected from the University of Jos staff quarter. A freshly collected leaves of *Eucalyptus camaldulensis* was air dried at room temperature and the dried leaves were then pounded into its powdered form with the aid of mortar and pestle. Hundred grams of the plant leaves powder was weighed using a weighing balance into a 500 ml conical flask and was constituted with 300 ml absolute diethyl ether and was left to stand for 48 hours and properly mixed for 1hour. The content was filtered using a Whattman filter paper placed on a Buchner funnel-flask using a vacuum pump. The residue was rinsed several times and then filtered using fresh diethyl ether to attain some level of exhaustive extraction. The filtrate was air-dried using an evaporating dish. The dried extract was harvested and stored in air-tight container for subsequent phytochemical analysis and anti-plasmodia assay in experimental mice.

### Preparation of Stock Solution

The stock solution was prepared just before use by dissolving the diethyl ether extract in 10% Dimethylsulfoxide (DMSO).

### Preparation of Experimental Animal

Albino mice were obtained from the animal house, University of Jos with an ethical clearance. They were feed with a standard animal feed and cared for, for about three weeks before the infection in other to get a good weight. The experimental mice were separated into six groups with three mice in each group and labeled differently base on the difference in body weight. Highly infected bloods observed under the microscope were obtained from the tail of an infected rat. The body weight of each mouse was taken using a weighing balance before inoculation of parasite. About 3mls of blood from the infected mice was collected in a beaker and mixed with 0.2 ml of normal saline. The blood was injected into the experimental albino mice intraperitoneally. The infectious *Plasmodium berghei* parasite with a strain NK65 was obtained from malaria research institute, Ibadan. The uninfected mice were inoculated intraperitoneally with 0.5ml parasite suspension.

### Experimental Design

The 18 mice were grouped into six (6) groups. Three treatment groups and three control groups (Positive, negative and normal control group). Each group has three mice.

The three treatments groups and the negative and positive control groups were infected with *Plasmodium berghei* parasite. The treatment groups were treated with 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of extracts. While the negative groups were left untreated.

Group A with 3 mice were infected and treated with 100 mg/kg of the extract per body weight per day.

Group B with 3 mice were infected and treated with 200 mg/kg of the extract per body weight per day.

Group C with 3 mice were also infected and treated with 300 mg/kg of the extract per body weight per day.

Group D (Positive control) with 3 mice were infected and treated with 25 mg/kg of standard drug (chloroquine) per day.

Group E (Negative control) with 3 mice were infected and untreated.

Group F (Normal control) with 3 mice were uninfected and untreated.

**Inoculation of Parasite into Experimental Mice**

The animals were inoculated using the method described by (Mann *et al.,* 2011). Infected mice were sacrificed and the blood sample was diluted with 0.2ml of normal saline, and the mixture was injected into the healthy mice intraperitoneally.

**Administration of Plant Extract and Standard Drug (Chloroquine)**

The mice that were infected with *Plasmodium berghei* were treated with diethyl ether extract of *eucalyptus camaldulensis* leaf intrapertoneally. And chloroquine was administered to the positive control group after 48 hours of inoculation daily for 10 days. The body weight of each mouse was taken using a weighing balance after administration.

**Parasitemia Determination**

Parasitemia was monitored by preparing a thin blood film. Two drops of blood collected from the tail of the mice were placed on one end of a labeled clean slide, then the edge of another slide is put in contact with the drop and the drop is allowed to bank evenly behind the speeded. Then the pusher slide is used to push the drop in smooth quick molten. The smear covers about half of the slide and air dry under a shade and dust free area. The film is then fixed with methanol for 2minutes and air dry, after then the fixed film is stained with giemsa stain and washed with clean water after about 10minutes. The number of parasites is then determined microscopically under x100 magnification with a drop of immersion oil placed at the tail end of the slide.

**Packed Cell Volume (PCV) Determination**

To determine the percentage of packed cell volume of blood sample, the blood sample is collected from the mice into an E.D.T.A container containing an anti-coagulant and a capillary tube were filled to two third with well mixed blood and one end of the tubes were sealed with plasticine. After that the filled blood was placed in the microhaematocrit centrifuge and spun at 12000 g for 5minutes and the spun tube is placed on a designed hematocrit reader and the PCV was read as percentage.

Packed cell volume (PCV) is the percentage of red blood cells in a circulating blood, when a known volume of blood is centrifuged at a constant speed for a constant time. A decreased in PCV generally means red blood cells loss from any variety of reasons like cell destruction, blood loss, and failure of bone marrow production. While an increased in PCV generally means dehydration or an abnormal increase in red blood cell production.

**Phytochemical Analysis of the Plant Extract**

Diethyl ether extract of *Eucalyptus camaldulensis leaf* was subjected to phytochemical screening to check for the presence or absence of plant secondary metabolites such as: Saponins, tannins, alkaloids, flavonoids, steroids and terpenes, cardiac glycosides, balsam, carbohydrates, phenols and resins according to the method of Trease and Evans, (1996) with slight modification.

**Test for alkaloids**

To 2 mls of extract, few drops of dragendorf's reagent were added to give orange colorations which indicated the presence of alkaloids.

**Test for flavonoids**

To 2 mls of the extract, few drops of 5 % lead acetate were added to give a cream light color which indicated the presence of flavonoids.

**Test for tannins**

To 2 mls of the extract, few drops of 10 % ferric chloride were added to give a deep bluish or greenish color which indicated the presence of tannins.

**Test for Saponins**

To 1ml of the extract, 4 mls of distilled water was added and shaken vigorously. Formation of froth indicated the presence of Saponins.

**Test for terpens and steroids**

To 1ml of the extract, 2mls of concentrated Sulphuric acid was added along-side of the test tube. Formation of reddish brown ring at the interphase indicated the presence of terpens and steroids.

**Test for cardiac glycosides (Salkowski's test)**

2 mls of the extract was dissolved in 2mls of chloroform and Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interphase indicated the presence of cardiac glycosides.

**General test for balsam**

3 drops of alcoholic ferric chloride were added to 2 mls of the extract. A dark green color formation indicated the presence of balsam.

**Test for carbohydrates**

5 drops of the extract were added to 2.0 mls of Benedict's reagent, placed on a hot plate for 5 minutes and was observed for the formation of brick red precipitation which indicated the presence of carbohydrates.

**Test for phenol**

To 2 mls of the extract, 2 mls of ferric chloride was added and observed for the formation of a deep bluish-green coloration which indicated the presence of phenol.

**Test for resins**

To 2 ml of the extract, 2 mls of acetic anhydride was added and drops of concentrated Sulphuric acid were added to observe for a violet color which indicated the presence of resins**.**

## 3. Results and discussion

**Percentage Yield and Phytochemical Screening**

The percentage yield of the Diethyl ether leaf extract of *Eucalyptus camaldulensis* was calculated to be 4.03 % and the result was given in Tables 1, 2 show the results obtained from the phytochemical screening of the Diethyl ether extract of powdered leaf of *Eucalyptus camaldulensis* and alkaloids, tannins, terpenes and steroids, balsam, resins, and cardiac glycosides were present.

**Parasitaemia Count**

Effect of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on parasitaemia level of *Plasmodium berghei* infected experimental mice were shown in Figure 1. Generally, compared to the negative control group all treatment groups had lower parasitaemia. Conversely the positive group which was treated with 25 mg/kg of chloroquine had much lower parasitaemia compared to the other groups (the treatment groups and the negative control group which was infected and untreated). Over the experimental period the level of parasitaemia was in a constant increase in negative control group and a constant decrease in positive control group. Among the treatment groups there was more suppressive effect of the plant extract on the parasitaemia level, treated with 200 mg/kg body weight.

**Packed Cell Volume**

Effects of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on packed cell volume of *Plasmodium berghei* infected experimental mice were shown in Figure 2. The packed cell volume of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extracts, 25 mg/kg chloroquine and the group infected but untreated decreased by 19 %, 22 %, 18 %, 4 % and 12 % respectively. Whereas the group uninfected and untreated increased by 1 %.

**Table 1.** Percentage yield of the diethyl ether leaf extract of *Eucalyptus camaldulensis*

| ***Eucalyptus camaldulensis*** | **Weight (g)** |
|---|---|
| Leaf extract | 100.00 |
| Diethyl ether extracts | 4.03 |
| Extract yield (%w/w) | 4.03 |

**Table 2.** Phytochemical constituent of the diethyl ether extract of *Eucalytus camaldulensis* leaf

| **METABOLITE** | **RESULT** |
|---|---|
| Alkaloid | + |
| Flavonoids | - |
| Tannins | + |
| Saponins | - |
| Terpenes and steroids | + |
| Cardiac glycosides | + |
| Balsam | + |
| Carbohydrate | - |
| Phenol | - |
| Resins | + |

**KEY**
- = absent
+ = present

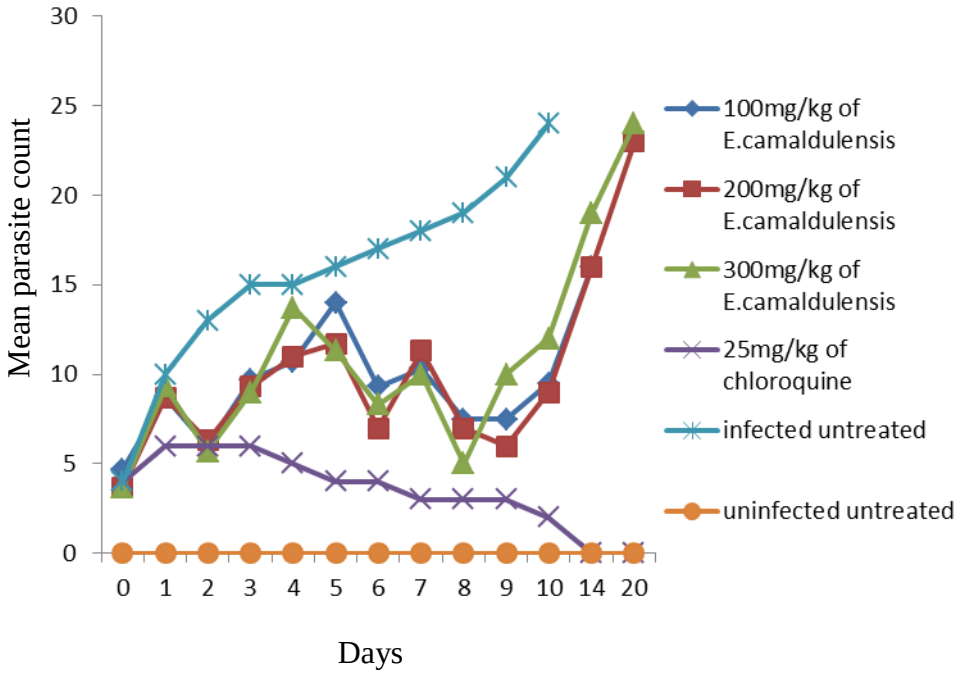


**Fig. 1.** Average mean parasitaemia count from day one of administration

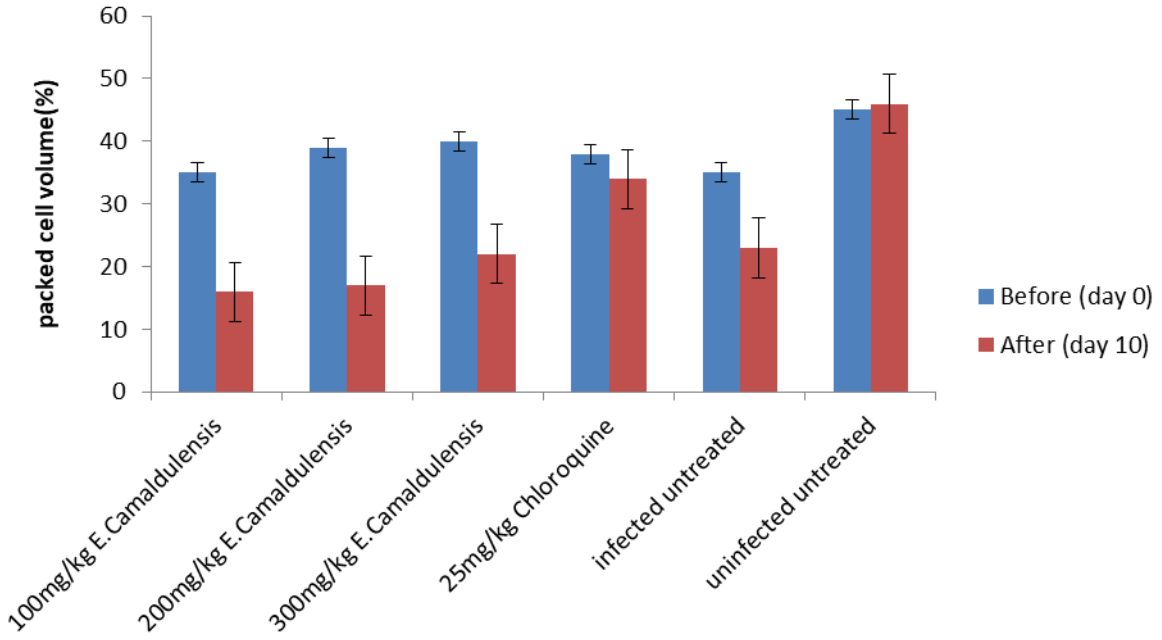


**Fig. 2.** The packed cell volume (PCV) of mice before inoculation of *Plasmodium berghei* and after administration

**Differences in Body Weight**

Effects of Diethyl ether leaf extract of *Eucalyptus camaldulensis* on the body weight of white albino mice infected with *Plasmodium berghei* were shown in Figure 3. The body weight of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extract, 25 mg/kg chloroquine and the group infected but untreated reduced by 1.47 g, 4.16 g, 1.16 g, 2.12 g and 1.2 g respectively whereas, the group uninfected and untreated increased by 5.2 g.

**Survival Time**

The death rates of the experimental albino mice were monitored daily after the inoculation of *Plasmodium berghei* as shown in Table 3. The groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg and the group infected but untreated died on day 17, 25, 25 and 14 respectively whereas, the groups treated with 25 mg/kg chloroquine and uninfected and untreated group lived beyond the experimental period.

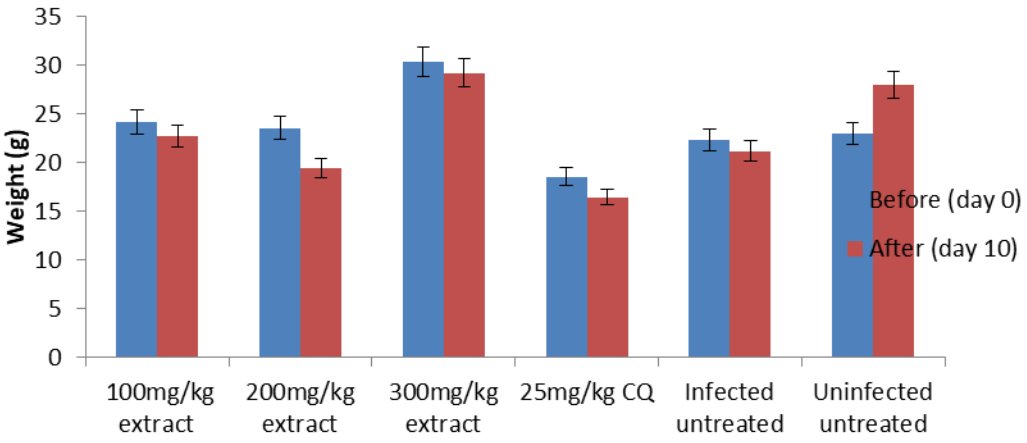


**Fig. 3.** Average mean weight of mice before inoculation of parasite and after administration

**Table 3.** Survival rate of experimental mice

| GROUPS | DAYS OF SURVIVAL POST-INFECTION |
|---|---|
| A (100mg/kg extract) | 1 7 |
| B (200mg/kg extract) | 2 5 |
| C (300mg/kg extract) | 2 5 |
| D (25mg/kg CQ) | Was cured and Survived all through experimental period |
| E (Infected Untreated) | 1 4 |
| F (Uninfected untreated) | Survived all through experimental period |

## 4. Conclusion

The percentage yield of the Diethyl ether leaf extract of *Eucalyptus camaldulensis* was calculated to be 4.03 % and the phytochemical screening of the Diethyl ether extract of powdered leaf of *Eucalyptus camaldulensis* are alkaloids, tannins, terpenes and steroids, balsam, resins, and cardiac glycosides. The body weight of the groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg extract, 25 mg/kg chloroquine and the group infected but untreated reduced by 1.47 g, 4.16 g, 1.16 g, 2.12 g and 1.2 g respectively whereas, the group uninfected and untreated increased by 5.2 g. The groups treated with 100 mg/kg, 200 mg/kg, 300 mg/kg and the group infected but untreated died on day 17, 25, 25 and 14 respectively whereas, the groups treated with 25 mg/kg chloroquine and uninfected and untreated lived beyond the experimental period.

The phytochemical screening of the crude diethyl ether leaf extract of *Eucalyptus camaldulensis* showed that the leaf contains useful phytochemicals which contributed to its anti-plasmodial activities in experimental mice. At the different concentrations of the diethyl ether extract dosage, the leaf of *Eucalyptus camaldulensis* showed varying degrees of treatment of the malaria parasite. Therefore, this shows that *Eucalyptus camaldulensis* has both curative and suppressive activities since at 200 mg/kg concentrations, the parasite load was seen to reduce and at 100 mg/kg and 300 mg/kg concentrations, the parasite was seen to also reduce but not as the former. Indicating that if treatment period is extended and a higher dosage concentration is administered, the parasite will be cleared completely. *Plasmodium berghei* was used in the prediction of treatment outcome and hence it was an appropriate parasite for this study (Dikasso et al., 2006).

Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice (Langhorne et al., 2002). So an ideal antimalarial ag ents obtained from plants are expected to prevent body weight loss in infected mice (Bantie et al., 2014). This research study present that the weight of the 200 mg/kg of the diethyl ether leaf extract of *Eucalyptus camaldulensis* significantly reduced with decrease in parasitaemia level when compared with the weight of the 100 mg/kg, 300 mg/kg and the group infected but untreated. While there is a significant increase in the weight of the group uninfected and untreated whereas, there is a decrease in the weight of the group treated with 25 mg/kg chloroquine.

## References

Bantie et al., 2014 – *Bantie, L., Assefa, S., Tekloehaimanot, T., Engidawork, E.* (2014). "In vivo antiplasmodial Activity of the crude leaf extract and solvent fractions of croton macrostachyus Hoscst. (Euphoribiaceae) against Plasmoduim berghei in mice". *BMC Complement Altern Med.*, 14: 79. DIO: 10.1186/1472-6882-14-79

Bloland et al., 2000 – *Bloland, P.B., Ettling, M., Meek, S.* (2000). Combination therapy for malaria in Africa: hype or hope. *Bull. World Health Organ.,* 78: 1378.

Dikasso et al., 2006 – *Dikasso, D., Mekonnen, E., Debella, A.* (2006). Antimalarial activity of Withania somnifera L. Dunal extracts in mice. *J. Ethiop. Med.,* 44: 279-285.

Igoli et al., 2005 – *Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A., Igoli, N.O.* (2005). Traditional medicine practice amongst the Igede people of Nigeria, part II. *Afr. J. Trad. CAM.,* 2(2): 134-152.

Langhorne et al., 2002 – *Langhorne, J., Quin, S.J., Sanni, L.A.* (2002). Mouse models of blood-stage malaria infections: immune responses and cytokines involved in protection and pathology. In: Perlmann P, Troye-Blomberg M, edictor. Malaria immunology. Stockholm: Karger Publisher, pp. 204-228.

Mann et al., 2011 – *Mann, A., Ifarajimi, O.R., Adewoye, A.T.* (2011). "in vivo antitrypanosomal effects of some ethnomedicinal plants from nupeland of north central Nigeria. *Afr. J. Tradit. Complement Altern. Med.,* 8(1): 15-21.

Muregi et al., 2003 – *Muregi, F.W., Chlabra, S.C., Njagi, E.N.M., Lang'at-Thoruur, C.C., Njue, W.M., Orago, A.S.S., Omar, S.A., Ndiege, I.O.* (2003). In vitro antiplasmodial activity of some plants used in Kissii, Kenya against malaria and their chloroquine potential effect. *J. Ethnopharmacol.,* 84: 235-239.

Titanji et al., 2008 – *Titanji, V.P.K., Zofou, D., Ngemeneya, M.N.* (2008). The Antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian Folk Medicine. *Afr. J. Trad. CAM.,* 5(3): 302-321.

Trease, Evans, 1996 – *Trease, G.E., Evans, W.C.* (1996). Trease and Evans pharmacognosy 14th edition w.b Ssuvnder company limited, London Pp. 191-293.

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# Selection and Study of Influence of Preparation "CMC" on the Process of Cooking of Blood

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**Abstract**

A study was carried out to identify a new structural fragment of coumarins from moldy clover (CMC) and to study the anticoagulant and toxicological characteristics.

The quantitative and qualitative composition of the coumarin mixture was studied using spectrophotometry, fluorescence analysis and high-performance liquid chromatography. Coumarins and coumarinic acids were identified by absorption spectra and retention time in comparison with standard samples. The specificity of the method of direct spectrophotometry was assessed from the absorption spectra of clover grass extracts, the standard solution of coumarin solutions of model coumarin mixtures. The general orientation of the change in the clotting process under the action of drugs was judged by the records of a thromboelastogram performed on a thromboelastography (Tromb-2).

The obtained fluorescence spectra as a function of the concentration of the extract showed a linear dependence of their intensity on the concentration, which made it possible to estimate the quantitative content of coumarins in CMC. The results show that the CMC really refers to the anticoagulants of indirect action, since the maximum CMC effect is manifested after 24 hours and is associated with a decrease in the content of procoagulants. By the effectiveness CMC is similar to the drug curantyl.

**Keywords:** coumarins, anticoagulants of indirect action, thromboelastography.

## 1. Introduction

One of the characteristic pharmacological properties of coumarin derivatives is the anticoagulant effect, the mechanism of which is to stop the normal formation of clotting factors-coagulation, which disrupt the synthesis in the liver of clotting factors-prothrombin and proconvertin, suppressing vitamin K-dependent synthesis of biologically active forms of calcium-dependent clotting factors blood II, VII, IX and X, as well as proteins C, S and Z in the liver (Shakhmatova et al., 2014).

At present, the number of isolated natural coumarins significantly exceeds 200 compounds that are found in both the free state and in the form of glycosides (Fedoseeva et al., 2013; Maksyutina et al., 1985). Structural fragments of coumarins have an anticoagulant effect. For example, dicumarol (*3,3'-methylene-bis-4-oxicoumarin*) interferes with blood clotting and causes painful bleeding in cattle caused by consumption of sweet clover (Knunyants, 1990). It was

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found that when feeding laboratory rats and mice with bait containing the extract of the moldy clover *Trifolieae* (EMC), depending on the dose, it caused severe bleeding in the animals for 5-8 days, which served as the basis for obtaining and studying its anticoagulant and toxicological characteristics.

The purpose of this work was to isolate a new structural fragment of coumarins from a moldy clover and study its anticoagulant and toxicological characteristics.

## 2. Materials and methods

To obtain the extract, clover specimens of the *Trifolieae* species were harvested at the time of flowering. Moldy clover was obtained leaving it in a cellophane bag in a dark place for 10 days. After the mold was formed, the moldy clover was crushed to a particle size of not more than 2 mm and subjected to extraction at a raw material / extractant ratio of 1: 100. The most complete extraction of coumarins (in free form and in the form of glycosides) was achieved when 45- 96 % ethyl alcohol was used both in the cold and during heating. At the same time, the yield of extractive substances was 32-36 % of the initial mass of the moldy clover.

To purify the amount of coumarins from the concomitant substances, the thick extract obtained after distillation of the extractant was treated with chloroform and a mixture of coumarins was recovered. After concentrating the extract, a mixture of coumarins in a crystalline state was obtained.

The quantitative and qualitative composition of the coumarin mixture was studied using spectrophotometry, fluorescence analysis and high-performance liquid chromatography, as described in (Sheluto et al., 2003).

Coumarins and coumarinic acids were identified by absorption spectra and retention time in comparison with standard samples. O-coumaric acid (Sigma, Cat.№. I2, 280-9), coumarin (Sigma, Cat.№.4261), scopolite (Sigma, Cat.№. S2500), umbelliferon (PhytoLab, Cat.№. 80098).

Based on the results of the research, the optimum conditions for preparation samples for the quantitative determination of coumarins in raw materials were determined.

The specificity of the method of direct spectrophotometry was assessed from the absorption spectra of clover grass extracts, the standard solution of coumarin solutions of model coumarin mixtures. The spectra were recorded on a spectrophotometer "SPEKOL 1300"

The experiments were carried out on 24 rats of males, females weighing 170.0 ± 15 g for 6 in each group. The study of the process of blood coagulation was carried out in dynamics after 24 hours and after 5 and 8 days from the moment of a single oral administration of the preparation "CMC" in doses of 0.04 and 0.06 mg/kg and curantyl in a dose of 50 mg/kg. Animal control group received distilled water orally in an equivalent volume.

To investigate the coagulation activity of the drugs, citrate plasma, taken from the blood of a rat prepared on sodium citrate 3.5 %, was used in a ratio of 1:9.

The general orientation of the change in the clotting process under the action of drugs was judged by the records of a thromboelastogram performed on a thromboelastography (Tromb-2) (Charnaya et al., 2010). The thromboelastogram included the following:

## 3. Results and discussion

Using spectrophotometry methods, it was found that in the UV spectra of the coumarin mixture, two characteristic maxima are observed for high-intensity dicoumarins in the range 220-380 nm, the first of which is of an oscillatory nature, and the second corresponds to the p-tt conjugation of the benzene ring (Figure 1).

Fluorescence analysis of the extract (a mixture of coumarins) showed that with a saturated bromine solution in an alkaline medium it was possible to identify them on the basis of equal excitation maxima (380 nm) and emission (480 nm) (Khabarov et al.,1980).

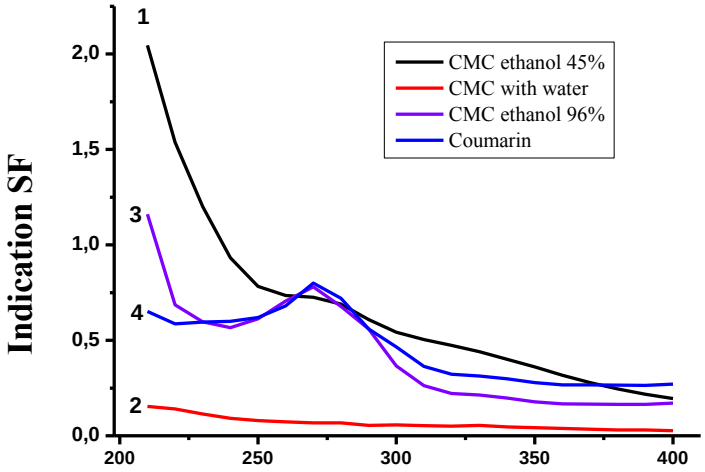


**Fig. 1.** Spectrophotometric readings of CMC and coumarin Electronic absorption spectrum of coumarin and CMC in 45-96 % alcohol extraction

The obtained fluorescence spectra as a function of the concentration of the extract showed a linear dependence of their intensity on the concentration, which made it possible to estimate the quantitative content of coumarins in this extract (Figure 2).

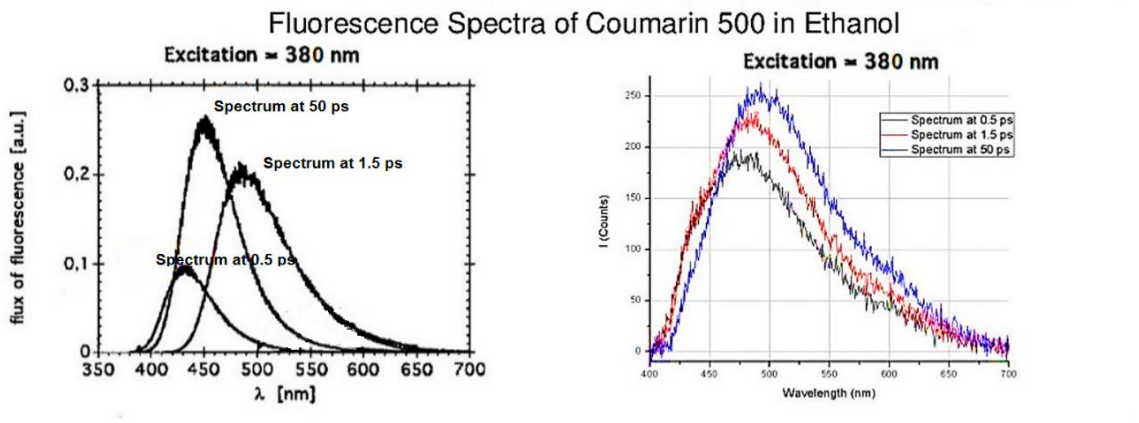


**Fig. 2.** Indications of intensity from the concentration of the fluorescence spectrum

Using HPLC in isocratic mode, a qualitative and quantitative analysis of the extract of moldy clover with standard dicumarol was performed – 15.22 min. (Figure 3). The amount of dicumarol in the CMC was calculated by comparing the peak areas in the chromatogram of the standard sample and in the test sample. As a result of the studies, it was found that in a dense extract the amount of dicumarol was 0.64 %, and in the crystalline fraction the sum of coumarins obtained with chloroform was 30.5 %.

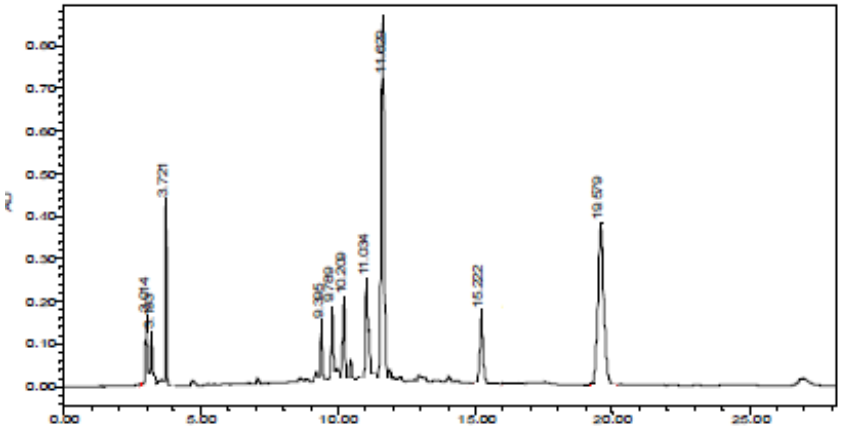


**Fig. 3.** Chromatogram CMC

Since the preparation CMC contains a mixture of dicumarols, which refers to vitamin K-dependent indirect anticoagulants, we investigated the effect on the blood coagulation process with its single administration compared with the effectiveness of curantyl.

The results of the effect of CMC on blood coagulation are shown in Table 1.

**Table 1.** Effect of the preparation "CMC" and curantyl on the indices of thromboelastograms of rats with oral administration (M ± m; n = 6)

| TEG indicators | Time of study through | | | |
|---|---|---|---|---|
| | control | 24 hours | 5 day | 8 day |
| Preparation CMC 0,04 mg/kg | | | | |
| Blood reaction time R, mm | 35±2,1 | 65±4,0* | 53±1,0* | 48±1,0* |
| Clot formation time, K, mm | 10±1,0 | 20±2,0* | 12±1,0 | 10±1,0 |
| Coagulation constant R + K, mm | 45±3,5 | 85±6,0* | 65±5,0* | 58±4,6 |
| The constant of using prothrombin R / K | 3,5±0,2 | 3,3±0,1 | 4,4±0,1* | 4,8±0,1* |
| Maximal amplitude MA, mm | 96±1,5 | 77±1,0* | 82±2,0* | 97±3,4 |
| Coagulation constant t, mm | 100±10 | 100±10 | 100±10 | 100±10 |
| The syneresis constant S, mm | 110±11 | 120±10 | 112±11 | 110±12 |
| Total clotting time T, mm | 145±14 | 185±14* | 175±15* | 158±13 |
| Hypercoagulation index Ci (MA / R + K) | 2,1±0,1 | 0,9±0,1* | 1,1±0,1* | 1,7±0,14* |
| Coefficient of elasticity of the bunch E, (MAX100 / 100-MA) | 2400±190 | 334±16,0* | 446±15,0* | 3233±14 |
| thrombohemorrhagic potential of ITP (E / S) | 21,8±2,0 | 2,8±0,2* | 4,0±0,3* | 29,4±2,1 |
| Preparation CMC 0,06 mg/kg | | | | |
| Blood reaction time R, mm | 35±2,1 | 67±4,0* | 50±1,0* | 48±1,0* |
| Clot formation time, K, mm | 10±1,0 | 17±1,1* | 15±1,0* | 13±1,0* |
| Coagulation constant R + K, mm | 45±3,5 | 84±3,0* | 63±3,0* | 61±3,0* |
| The constant of using prothrombin R / K | 3,5±0,2 | 3,59±0,1 | 3,3±0,1 | 4,0±0,1 |
| Maximal amplitude MA, mm | 96±1,5 | 80±1,0* | 82±1,0* | 93±1, |
| Coagulation constant t, mm | 100±10 | 100±10 | 100±10 | 100±10 |
| The syneresis constant S, mm | 110±11 | 117±10 | 115±10 | 112±10 |
| Total clotting time T, mm | 145±14 | 184±14* | 163±15* | 164±16* |
| Hypercoagulation index Ci (MA / R + K) | 2,1±0,1 | 0,9±0,1* | 1,4±1,0* | 1,55±1,0* |
| Coefficient of elasticity of the bunch E, (MAX100 / 100-MA) | 2400±190 | 400±20* | 456±30* | 1329±100* |

| | | | | |
|---|---|---|---|---|
| thrombohemorrhagic potential of ITP (E / S) | 21,8±2,0 | 3,4±1,4 | 4,0±2,5 | 11,9±1,1 |
| Curantyl, 50 mg/kg | | | | |
| Blood reaction time R, mm | 35±2,1 | 72±3,2 | 61±6,6* | 53±5,5 |
| Clot formation time, K, mm | 10±1,0 | 28±2,0* | 39±2,2* | 31±2,2* |
| Coagulation constant R + K, mm | 45±3,5 | 100±8,3 | 100±11* | 94±6.0* |
| The constant of using prothrombin R / K | 3,5±0,2 | 2,6±0,2 | 1,7±0,2 | 2,0±0,2 |
| Maximal amplitude MA, mm | 96±1,5 | 82±2,0 | 77±1,0* | 87±2,0 |
| Coagulation constant t, mm | 100±10 | 105±10 | 105±10 | 105±10 |
| The syneresis constant S, mm | 110±11 | 132±12 | 144±13 | 136±12 |
| Total clotting time T, mm | 145±14 | 232±16* | 205±16* | 199±16* |
| Hypercoagulation index Ci (MA / R + K) | 2,1±0,1 | 0,83±0,6 | 0,77±0,06 * | 0,6±0,04 * |
| Coefficient of elasticity of the bunch E, (MAX100 / 100-MA) | 2400±190 | 456±30 | 334±16* | 669±12* |
| thrombohemorrhagic potential of ITP (E / S) | 21,8±2,0 | 3,5±0,1* | 2,3±0,2* | 4,9±0,1* |

* P≤ 0,05 in relation to the control

As can be seen from the data in Table 1, 24 hours after the administration of CMC, at a dose of 0.04 mg/kg on a thromboelastogram, we observed hypocoagulation, which is expressed by an increase in the R, K, and R + K values by a factor of 2, prothrombin (R) and the concentration of thrombin formed and the amount of fibrinogen (K). Also, the Ci-index of hypercoagulation decreased 2-fold.

The maximum effect of the drug on the indicators E and ITP, where the coefficient of elasticity of the coagulum E decreases from 2400-190 to 334 16.0 or 7 times, and the thrombohemorrhagic potential of the ITPs is 21.8-2.0 to 2.8-8.2 or 7.8 times.

Gradually, the effect of the drug decreases, respectively, after 8 days is close to control.

An increase in the therapeutic dose of CMC to 0.06 mg/kg does not increase the effect after 24 hours, but it increases the duration of the drug.

As can be seen from the data in Table 1, such parameters as the elasticity coefficient of the clot E and the thrombohemorrhagic potential of the ITP, remained 8 days after the administration of the drug, decreased 2-fold with respect to the control group of animals (respectively, from 2400±190 to 1329±100 21.8±2.0 to 11.9±1.1).

As can be seen from the data in Table 1, a single administration of quarantine after 24 hours resulted in an increase in the reaction time of blood R by 100%. The K indicator increased by 69.6 %, and the MA maximum amplitude decreased by 13.6 %, which indicates a decrease in the formation of both thrombin and fibrinogen. Moreover, the hypercoagulation index Ci decreased by 52 %, the elasticity of the clot E by 33 %, and the thrombohemorrhagic potential index by 46 %.

After 8 days of the recovery period after the abolition of the curantyl, the blood coagulation indexes studied remained significantly altered towards hypocoagulation, although the effect was somewhat weaker.

## 4. Conclusion

The conducted studies made it possible to conclude that CMC is indeed an anticoagulant of indirect action, since the maximum effect of the drug is manifested in 24 hours and is associated with a decrease in the content of procoagulants. It is effective in comparison with the drug curantyl.

## References

Charnaya et al., 2010 – *Charnaya, M.A., Morozov, Yu. A., Gladysheva, V.G.* (2010). Use of the thromboelastography method for diagnosis and selection of tactics for correction of hemostasis system disorders in the cardiosurgical clinic. *Vestnik anetenziologii i reanimatologii.* №1. Pp. 28-33.

Fedoseeva et al., 2013 – *Fedoseeva, L.M., Kharlampovich, T.A.* (2013). The study of some water-soluble compounds of the sweet clover of the medicinal herb (*Melilotus officinalis L.*). Chemistry of plant raw materials. Barnaul, Is. 2. Pp. 153-157.

Khabarov et al., 1980 – *Khabarov, A.A., Khabarova, N.P.* (1980). Tez. Doc. III Vses. Congress of Pharmacists, Chisinau. P. 278.

Knunyants, 1990 – Chemical Encyclopedia. Redkol.: *Knunyants I.L.* and others. Moscow: Soviet Encyclopedia, 1990. Vol. 2 (Daf-Med). P. 671.

Maksyutina et al., 1985 – *Maksyutina, N.P., Komisarenko, N.F., Prokopenko, A.P.* (1985). Herbal medicines development of methods for quantifying the amount of coumarins in the sweet clover of medicinal grass (*melilotus officinalis L*). Kiev, P. 280.

Shakhmatova et al., 2014 – *Shakhmatova, O.O., Panchenko, E.P.* (2014). Echo Ateroshkol. Atherothrombosis. Number 1. P. 98-104.

Sheluto et al., 2003 – *Sheluto, V.L., Buzuk, G.N., Konopleva, M.M., Lovchinovsky, Yu. O., Ed. Sheluto, B.L.* (2003). Pharmacognosy. Tutorial. Vitebsk: Vitebsk State Medical University, 2003. P. 490.

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# Activity of Chloroform Extract of *Eucalyptus camaldulensis* Root against *Plasmodium Berghei* in vivo

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**Abstract**

The work was designed to investigate the anti-malarial activity of chloroform root extract of *Eucalyptus camaldulensis* on *Plasmodium berghei* NK65 strain infection *in vivo*. Eighteen mice were intraperitoneal infected with chloroquine sensitive *P. berghei* strain and shared into 6 equal groups. Groups A, B, and C were treated, after infection, with 100, 200 and 300 mg extract/kg body weight of mouse respectively while group D was treated with 25 mg chloroquine/kg body weight. Group E mice were infected and administered only normal saline (negative control), and group F was neither infected nor treated. Phytochemical constituents of the plant extract were evaluated. The three concentrations of the extract resulted in reduced parasitemia, although the 200 mg/kg administered to group B had more effect than the 300mg/kg and 100 mg/kg administered to group C and A respectively. The highest activity was observed in the chloroquine group (positive control group). Also, at doses of 100 mg/kg, 200 mg/kg and 300 mg/kg, the extract produced increase in body weight and life span as compared to mice in the negative control group. At doses of 100mg/kg and 300mg/kg, the extract produced increase in PCV of the infected mice as compared to mice in the negative control group. Phytochemical screening showed that the leaf extract contains alkaloids, Balsam, Resin, cardiac glycosides and terpenes and steroids. The chloroform root extract of *Eucalyptus camaldulensis* presented a transient effect on *Plasmodium infection* in mice and so justifies the use of the plant as part of native desertion against malaria.

**Keywords**: *Plasmodium berghei;* malaria*; Eucalyptus camaldulensis.*

## 1. Introduction

Malaria is a vector–borne infectious disease caused by eukaryotic protists of the genus Plasmodium. There are four types of *Plasmodium* species that caused malaria namely, *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale*, and *Plasmodium Malariae*. It is a disease transmitted by infected female Anopheles mosquito (Toure et al., 2004). A *Plasmodium* Spp depends on two hosts to complete its life cycle, a female anopheles mosquito and a human. The disease is widely spread in tropical and sub-tropical regions, including most of sub-Saharan Africa, Asia and America. Malaria is prevalent in these regions because of the significant amount of rainfall, warm temperatures, and high humidity, along with stagnant waters that provide the mosquitoes with the environment needed for continuous breeding (Prothero; Mansall, 1999).

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It is estimated that about 81 % of all malaria deaths in the world occur in African region. Majority of infections in Africa is caused by *Plasmodium falciparum*, the most lethal of the four human malaria parasites. About 655,000 people die of malaria, 91 % in African region, and 86 % of this are children under the age of five. Malaria is a risk of 97 % of Nigeria's population. There are estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria (WHO, 2016). Nigeria's ministry of health reported in April 2004 that malaria is responsible for one out of ten deaths in pregnant women and cost the federal government over one billion naira annually. The report added that Nigeria is often associated with poverty which is the major hindrance to economic development, despite the effort made to reduce the transmission; there has been little change in areas at risk of this disease since 1992. The precise statistics are unknown because many cases occur in rural areas where people do not have access to the hospital or the means to afford health care (Hay et al., 2008). Malaria has infected humans for over 50,000 years and may have been a human pathogen for the entire history of humans (Joy et al., 2003).

Anti-malarial drugs have been used in various ways to prevent or treat malaria infection in the resident populations of malaria-endemic' areas for nearly 100 years (Greenwood, 2004). However the continual resistance of the parasite especially *Plasmodium falciparum* to currently used anti-malarial drugs make it imperative to search for newer and more effective therapies. Quinin was used in the seventeenth century as a prophylactic against malaria followed by the development of quinacrine, chloroquin, and primaquin were used in the twentieth century to reduce the reliance on quinine (WHO, 2006). The World health organization initiated the based combination therapy, (artemisinin), obtained from *Artemisia annua* (a plant in china), and quinine to treat malarial infection (WHO, 2007).

*Eucalyptus camaldulensis* is found in many parts of the world. It has been use in Australia as a source of honey (Lupo, Eisikiowitch, 1989). The leaves are used to treat trypanosomiasis (Kabiru et al., 2012). Phytochemical screening revealed the presence of terpenes, steroids, tannins, alkaloids, and fatty acid. It possess anti-inflamatory, analgesic, antimicrobial, antiparasitic, antioxidant, antidiabetic, anti-insecticide, repellent, dermatological and anti cancer effect (Arl-Snafi, 2015). Therefore, the rat was studied against malaria parasite in this study.

## 2. Materials and methods

### Plant Collection, Preparation and Extraction

Roots of *Eucalyptus camaldulensis* were collected from university of Jos senior staff quaters, and were authenticated by a Botanist in the College of Forestry Jos.The roots were washed with clean-water, air dried to constant weight and milled using a mechanical grinder. Extraction of plant material was performed by soxhlet apparatus (Sigma-Aldrich, USA) using chloroform. The extract was stored in a Refrigerator until required. About 100 g of the extract was put into a conical flask and soaked in 500 ml of 99 % chloroform. This was then left for 24 hours. The mixture was filtered using a white cloth and Whatmann filter paper. The filtrate was then kept in the laboratory oven at 40$^{o}$ C to dry. The dried extract was used for phytochemical analysis and anti-plasmodial assay in experimental albino mice.

### Phytochemical Analysis of the Plant Extracts

The chloroform extract was subjected to phytochemical screening to detect the presence or absence of plant secondary metabolites: alkaloids, Balsam, Resins, terpenes and steroids, and cardiac glycosides according to the method of Trease and Evans (1987).

### Parasite Inoculums

*Plasmodium berghei* infected erythrocytes were obtained from a donor-infected mouse at malaria research institute, Ibadan and maintained at animal farm University of Jos, Plateau state, Nigeria. The inoculum was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

### Experimental Animal and Curative Test

The eighteen (18) albino mice weighing between 18.6-25.6 g used in this study were obtained from the animal house of University of Jos, Plateau State, Nigeria. They were kept in plastic cages

with saw dust bed and given standard laboratory chore and water. They were then allowed to acclimatize for two weeks to their new environment before the initiation of the experiments. In order to evaluate the antimalarial potential of the crude extract, methods described (Akuodor, Idris, 2011; Ryley, Peters, 1995) in literature were adopted. Each mouse in the treatment group was inoculated intraperitoneal with infected blood suspension (0.3 ml) containing *Plasmodium berghei* parasitised red blood cells on day zero. Groups A, B, and C were dosed once daily for ten days with 100, 200 and 300 mg/kg/day of the chloroform root extract respectively. Chloroquine diphosphate (25 mg/kg body weight/day) was administered to group D mice and 0.2 ml normal saline to group E mice (negative control group). All treatments were orally done for ten days from when parasites were first seen in the infected animal blood.

**Parasitemia Count**

On each day of treatment, a drop of blood was collected from the tail of each infected mouse for parasitemia screening. The blood collected was placed on a slide and smeared to make a thick film, fixed with methanol and stained with Giemsa stain and air-dried. The film was microscopically viewed by adding a drop of immersion oil and viewed under x100 magnification of the microscope. The parasitemia density was examined by counting the parasitized red blood cell.

**Determination of Packed Cell Volume**

Capillary tubes were filled with blood to about 1 cm or two-third (2/3) of its length and the vacant end of each sealed with plasticin to protect the blood from spilling. The tubes were placed in haematocrit centrifuge with sealed side towards the periphery and then centrifuge for 5 minutes. The packed cell volume was read directly from haematocrit reader.

## 3. Results

**Extract Yield**

The percentage yield of the leaf extract is shown in Table 1. The yield of chloroform root extract of *E. camaldulensis* was 16.5 %. Table 2 shows the result of phytochemical composition of chloroform root extract of *Eucalyptus camaldulensis*. The results revealed the presence of alkaloids, Balsam, Resins, cardiac glycosides, Terpenes and steroids.

**Table 1.** Percentage yield of chloroform root extract

| | |
|---|---|
| Root powder | 100.00 |
| Chloroform extract | 16.50 |
| Extract yield (% w/w) | 16.50 |

**Table 2.** Phytochemical composition

| phytochemicals | Inference |
|---|---|
| Alkaloid | + |
| Flavonoids | - |
| Tannins | - |
| Saponins | - |
| Terpenes and steroids | + |
| Cardiac glycosides | + |
| Balsam | + |
| Carbohydrates | - |
| Phenols | - |
| Resins | + |

*Key: (-) absent, (+) present*

**Parasitaemia Count**

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with Chloroform root extract of *Eucalyptus camaldulensis* are shown in Figure 1. The average daily

parasitaemia of infected mice treated, 100, 200, 300 mg/kg of root extract of *Eucalyptus camaldulensis* and chloroquine (25 mg/kg) reduced when compared with control group, although the 200 mg/kg is more effective followed by the 100 then 300 mg/kg. The highest activity was observed for the standard group (chloroquine).

**Body Weight**

Effect of chloroform roots extract of *Eucalyptus camaldulensis* on body weight of *Plasmodium berghei* infected mice is shown in Figure 2. The body weight of the infected but untreated mice showed a decrease in body weight. Those treated with, 100, 200, chloroquine, and 300 mg/kg respectively showed significant ($P < 0.05$) increase in body weight after 10days post treatment when compare to the infected but untreated group (negative control).

**Packed Cell Volume**

Effect of chloroform root extract of *Eucalyptus camaldulensis* on PCV of *Plasmodium berghei* infected mice is shown in Figure 3. The PCV of *P. berghei* infected untreated mice gave about % reduced. Those infected and treated with 300, 200, and 100mg/kg of *Eucalyptus camaldulensis* root respectively as well as those treated with 25 mg/kg chloroquine showed various effects on the PCV that are better than that in negative control group. On the other hand, the un-infected untreated mice showed significant increase in PCV after 10 days of treatment.

**Effect of *Eucalyptus camaldulensis*' root on survival time**

Figure 4 shows the survival time of the mice treated with the various concentrations of the crude extract and those infected, but untreated. The normal control, positive control and the group treated with 100 mg/kg lived beyond the experimental period of twenty one days; the group treated with 300 mg/kg lived for twenty days; the group treated with 200 mg/kg lived for sixteen days, while the negative control lived for fifteen days.

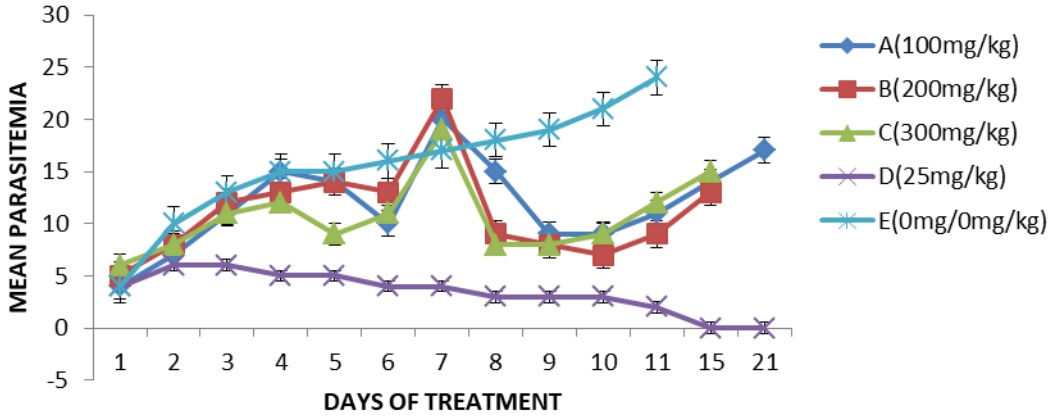


**Fig. 1.** The parasitaemia levels after treatment with chloroform root extract of *Eucalyptus camaldulensis*

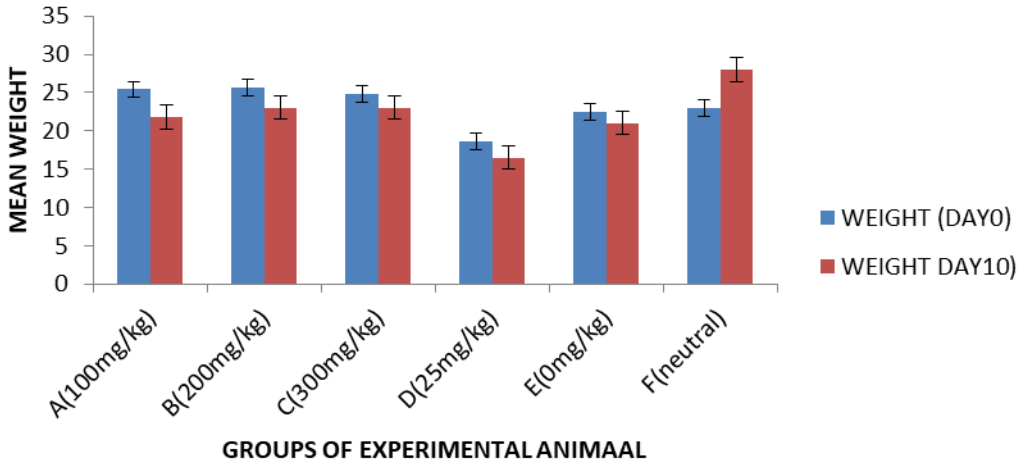


**Fig. 2.** The mean weight of *Plasmodium berghei* infected mice treated with chloroform root extract of *Eucalyptus camaldulensis*

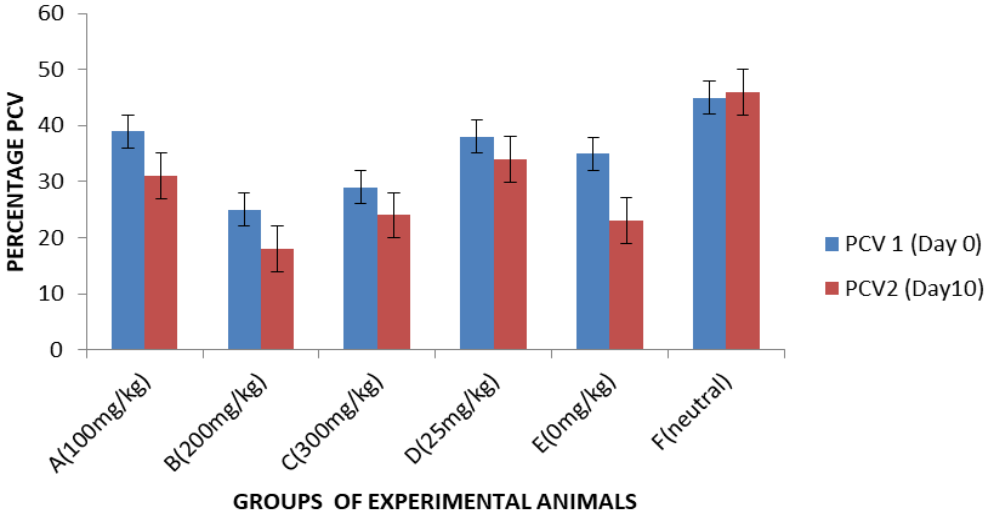


**Fig. 3.** The mean packed cell volume of *Plasmodium berghei* infected mice treated with chloroform root extract of *Eucalyptus camaldulensis*

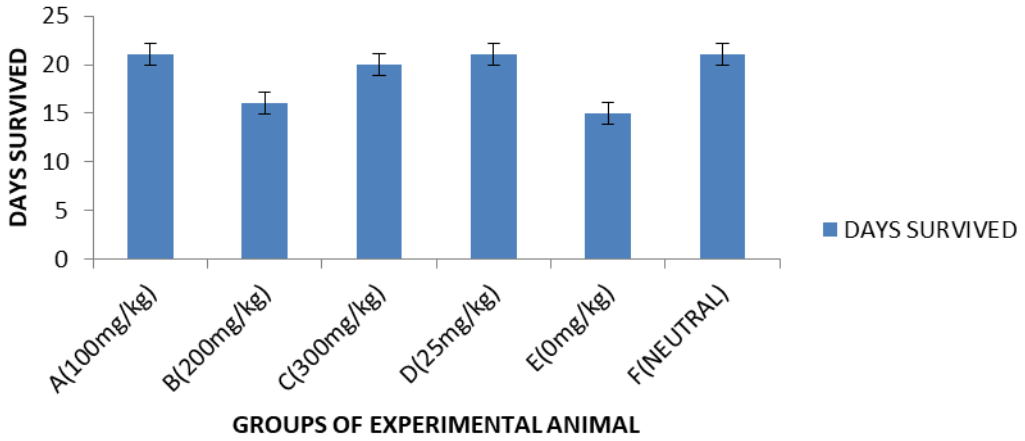


**Fig. 4.** The survival time after treatment with chloroform root extract of *Eucalyptus camaldulensis*

## 4. Discussion

Plants used in treatment of diseases are said to contain active phytochemicals some of which are responsible for the plants' characteristic odours, pugencies and colour while others give virtue as food, medicinal or poison (Evans, Evans, 2002). The phytochemical screening of the extract revealed that *Eucalyptus camaldulensis's root* contained some active chemical compounds such as Alkaloids, Flavonoids, Resins, Balsam, cardiac glycoside, Terpenes and Steroids. The presence of secondary metabolites in plants produced some biological activity in man and animals and it is responsible for their used as drugs (Evans, Evans, 2002) and therefore explains its traditional use as health remedy. Secondary metabolites in plants confer protection against bacterial, fungal and pesticidal attacks and thus, are responsible for the exertion of antimicrobial activity against some microorganisms (Marjorie, 1999). Flavonoids have been reported to have exhibited significant *in vitro* antimalarial activity against *P. Falciparum* (Wanauppathamkul, Yuthavong, 1998). This could justify the antimalarial activities exhibited by the plant extract. The 200mg/kg administered to group B has more effect than the 300mg/kg and 100mg/kg administered to group C and A respectively. After treating the curative groups for ten days, parasitemia level was checked on day 11, 15, and 21, and it was observed that the parasitemia level increased in the curative group that means there was a relapse. *Eucalyptus camaldulensis* leaf extract was found in previous studies to be effective against Trypanosoma *brucei* (Kabiru et al., 2012) but from this study it was not effective against *Plasmodium berghei*. The finding in this study did not agree with earlier reports of studies using different extract. Antiplasmodial activity was observed in the ethyl acetate crude extract of C*arica papaya* against *P. falciparum* (Melariri et al., 2011). Unlike *Carica papaya* which was found to be effective against *Plasmodium berghei* in previous study (Longdet, Adoga, 2017), chloroform root extract of *Eucalyptus camaldulensis* has only transient effect on *Plasmodium berghei.* Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* (Ajaiyeoba et al., 2006). Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice (Langhorne et al., 2002). So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice (Bantie et al., 2014). The extract of *E. Camaldulensis* at 100, 200, and 300 mg/kg body weight decreased the weight of the infected mice following ten days of administration after infection. The chloroform root extract of *Eucalyptus camaldulensis* at 200 and 300 mg/kg body weight *prevented* weight loss associated with increase in parasitemia level by 10.2 % and 7.3 % respectively when compared with the positive control. This is an indication of ameliorating potentials of the plant extract on weight loss. Extracts of *Eucalyptus camaldulensis* decrease the level of PCV in infected mice, this is expected as anemia is one of the general features of malaria in infected mice. At 100, 200, and 300mg/kg body weight, the PCV level reduced by 20.5 %, 28 %, and 17.2 % when compared with the negative control group which reduced the PCV level by 34.3 %, we can say that the extract helped in preventing anemia associated with increase in parasitemia.

## 5. Conclusion

This indicates that *Eucalyptus camalduensis* contains important phyto-constituents that could be implicated in the observed antimalarial effect of the plant. However, the active compound (s) known to give this observed activity need to be identified.

## References

Ajaiyeoba et al., 2006 – *Ajaiyeoba E., Falade M., Ogbole O., Okpako L., Akinboye D.* (2006). *In vivo* antimalarial andcytotoxic properties of *Annonasenegalensis* extract. *African Journal of Traditional Medicine*, 3(1): 137-141.

Akuodor, Idris, 2011 – *Akuodor, G.C., Idris, U.I.* (2011). Anti-nociceptive, anti – inflammatory and antipyretic effect of the methanolic extract of *Bombax buonopozense* leaves in rats and mice. *Afr. J. Biotechnology:* 10: 3191 3196.

Al-Snafi, 2015 – *Al-Snafi A.E.* (2015). Therapeutic properties of medicinal plants: a review of their detoxification capacity and protective effects. *Asian Journal of Pharmaceutical Science & Technology*, 5(4): 257-270.

Bantie et al., 2014 – *Bantie L., Assefa S., Teklehaimanot T., Engidawork E.* (2014). *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus*

Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. *BMC Complement Altern Med.,* 14: 79. DOI: 10.1186/1472-6882-14-79

Evans, Evans, 2002 – *Evans, W.C., Evans, T.* (2002). Pharmalognosy (15 th edition). W.B Saunders Company LTD. London, 191-393.

Greenwood, 2004 – *Greenwood, B.* (2004). The use of anti-malaria drugs to prevent malaria in the population of malaria-endemicareas. *A.M.J. Trop .Med .Hug.,* 7 (1).

Hay et al., 2008 – *Hay, S.I., Smith, D.L., Snow, R.W. (*2008). Measuring malaria endemicity from intense to interrupted transmission. *The Lancet Infectious Diseases,* 8: 369-378.

Joy et al., 2003 – *Joy, D.A., Feng, X., Mu, J., Furuya, T., Chotivanich, K., Krettli, A.U., Ho, M., Wang, A., White, N.J, Suh, E., Beerli, P., Su, X.Z.* (2003). Early origin and recent expansion of *Plasmodium falciparum*. *Science,* 300(5617): 31821.

Kabiru et al., 2012 – *Kabiru, Y.A., Ogbadoyi, E.O., Okongun, J.I., Gbodi, T.A., Makua, H.A.* (2012). "Department of Biochemistry, Trypanosomiasis and malaria research unit, Federal University of Technology Minna, Nigeria".

Langhorne et al., 2002 – *Langhorne J., Quin S.J., Sanni L.A.* (2002). Mouse models of blood-stage malaria infections: Immune responses and cytokines involvedin protection and pathology. In: Perlmann P, Troye-Blomberg M, editor. Malaria Immunology. Stockholm: Karger Publisher; 204–228.

Longdet, Adoga, 2017 – *Longdet, Ishaya. Y., Adoga, Emmanuel. A.* (2017). Effect of methanolic leaf extract of *Carica papaya* on *Plasmodium berghei* infection on albino mice. *Department of Biochemistry, Faculty of Medical Sciences, University of Jos, Plateau State, Nigeria*. DOI: 10.9734/EJMP/2017/34698

Lupo, Eisikiowitch, 1989 – *Lupo, A., Eisikiowitch, D.* (1989). *Eucalyptus erythrocoris*: a source of nectar and pollen for honey bes in Isreal. Department of Botany, the Goerge S wise oflife science.

Marjorie, 1999 – *Marjorie, M.C.* (1999). Plant product as anti-microbial agents. *Clin. Microbial, Rev.,* 12(4): 564.582.

Melariri et al., 2011 – *Melariri, P., Campbell, W., Etusim, P., Smith, P.* (2011). Antiplasmodial properties and bioassayguided fractionation of ethyl acetate extracts from *Carica papaya* leaves. *Journal of Parasitology Research*. DOI: http://dx.doi.Org/10.1155/2011/104954

Prothero, Mansell, 1999 – *Prothero, R., Mansell, W.* (1999). Malaria, forests and people in southeast Asia. *Singapore Journal of Tropical Geography,* 20(1): 76-85.

Ryley, Peters, 1995 – *Ryley, J., Peters, W.* (1995). The antimalarial activity of some quinoline esters. Ann Trop Med Parasitology, 84(22): 209–2.

Toure et al., 2004 – *Toure, Y.T., Oduola, A.M., Morel, C.M.* (2004). The *Anopheles gambiae* genome: next steps for malaria vector control. *Trends in Parasitology,* 20: 142-149.

Trease, Evans, 1987 – *Trease E., Evans W.C.* (1987). Pharmacognosy. Billiaretindall London. 13th Edition; 21: 61-62.

Wanauppathamkul, Yuthavong, 1998 – *Wanauppathamkul, S., Yuthavong, Y.* (1998). Antimalarial principles from *Artemisia indica*. *Journal of Natural Products*, 61: 1146-1147.

World Health Organization, 2007 – World Health Organization (2007). Facts on ACTs (artemisinin-based Combination therapies). Geneva Available at: http://www .rollbackmalaria.org/psm/acts.html.

World Health Organisation, 2006 – World Health Organisation (2006). Guidelines for the treatment of malaria.

World Health Organization, 2016 – World Health Organization (2016). Factshee: World Malaria Report. [Electronic resource]. URL: http: //www.who.int/malaria/media/world-malaria-report-2016/en/ (accessed on June 22, 2017).