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RESEARCH ARTICLE

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## Comparative studies on phytochemicals screening and antimicrobial activities of shed leaves, fresh leaves and roots of *Eucalyptus Camaldulensis* in Zaria, Nigeria

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### ABSTRACT

Medicinal plants are extensively used in traditional medicine to cure various infectious diseases in human. The extract of leaves and root of *Eucalyptus camaldulensis* were screened phytochemically for the presence of secondary metabolites and the antibacterial properties. The methanol extracts of fresh leaf, shed leaf and root of *Eucalyptus camaldulensis* were studied against three isolated organisms *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. The result of phytochemical screening showed that extract of fresh leaf, shed leaf contain anthraquinones, carbohydrate, cardiac glycoside, flavonoid, saponins, triterpenes while the root extract lack all these but contain only carbohydrate. Fresh leaf and shed leaf of *E. camaldulensis* were active against all the bacteria isolated and root extract was active against only gram positive (*Staphylococcus aureus*). The ability of the crude extracts of the leaves to inhibit the growth of bacteria is an indication of its broad spectrum. The antibacterial activity of the leaves extracts of *E. camaldulensis* can be attributed to the action of the phytochemical compounds they contain. There was no significant difference in the antimicrobial activity of the green leaf and shed leaf extracts but they were significantly different from the root extract of *E. camaldulensis*. The results of this study support the traditional use of *Eucalyptus camaldulensis* leaves as an antibacterial agent.

**Key words:** *Eucalyptus camaldulensis*, secondary metabolites, antibacterial properties, methanol extracts, shed leaf, fresh leaf.

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### INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [21]. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry [14].

The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plant [6]. It has been reported that the antimicrobial activities of medicinal plants can be due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids [10]. In this sense, natural medicine has given special importance to the search for antimicrobial agents as a consequence of the increasing resistance of bacteria to commercial antibiotics [24].

Many plants with medicinal properties have been reported multiple times by host of researchers. Plants in the family Fabaceae have been reported to have phytochemicals with antimicrobial agents used in treatments of infectious diseases like malaria, pneumonia [16]. Fallen leaves of *Carica papaya* were used as abortifacients [21].

According to an estimate by World Health Organization (WHO), 80% of the people in developing countries rely cheaply on the traditional medicine for their active principles [9].

Bacteria are responsible for well-known diseases such as tuberculosis, syphilis, tetanus, cholera and many types of pneumonia. It has been verified that 93% of wound infections are caused by *Staphylococcus aureus* [18, 19]. In other cases, microorganisms taken from bacteriemias, gastroenteritis and urinary tract infections are all caused by *Escherichia coli*, a pathogen widely dispersed in nature that produces the major part of child diarrheas [11].

The *Eucalyptus* genus comprises over 500 species of aromatic trees and shrubs. In traditional medicine essential oil of *Eucalyptus camaldulensis* has been applied for the treatment of respiratory tract disorders, cold, chest pain, coughs and infections.

This study was carried out to assess the phytochemicals screening and antimicrobial activities of fresh leaves, shed leaves and roots of *Eucalyptus camaldulensis*, with the view of investigating the antimicrobial activities of shed leaves as compared with fresh leaves and root samples.

## MATERIALS AND METHODS

### Study Area

The research was carried out in the Department of Biological Sciences, Department of Microbiology and Department of Pharmacognosy, Ahmadu Bello University, Zaria, Nigeria. Zaria is located in Northern Guinea Savannah zone of Nigeria with coordinates (11<sup>0</sup>3'N; 7<sup>0</sup>42'E).

### Collection and Identification of Plant Materials

The fresh leaves, shed leaves and root of *Eucalyptus camaldulensis* were collected at Faculty of Education A.B.U Samaru, Zaria. These plant materials were taken to the herbarium at the Department of Biological Sciences, A.B.U., Zaria for proper identification with scientific name.

### Processing of Plant Materials

The identified plant parts were washed with tap water and air-dried for 2 weeks in well a ventilated room. The purpose of drying is to prevent ultraviolet rays from destroying the active ingredients in the fresh leaves, shade leaves and root; this also removes or reduces water content to a larger extent. The dried parts were chopped into pieces, milled into fine powder by pounding manually with a clean and sterile pestle and mortar in the Department of Pharmacognosy and Drug Development, Faculty of Pharmacy, A.B.U., Zaria. The dried powdered samples were collected into sterile cellophane bags and labeled to prevent mix up. The samples were kept in cool dry place till further use.

### Test Microorganisms

The laboratory isolates of three bacteria species which include two gram negative and one gram positive were obtained from Department of Microbiology, Faculty of Science, A.B.U., Samaru, Zaria. The organisms used in this study were *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*.

### Experimental Design

#### Extraction Process

1000ml of methanol was added to 100 g each of the dried powdered samples in a flask. Each of the soaked sample was stirred, sealed with aluminum foil and allowed to stand for 72 hrs. The content was then filtered with Whatman No. 1 filter paper. The filtrates were concentrated using rotary evaporator at 40°C. The extracts were stored in a universal bottle and refrigerated at 4°C prior to use [17].

$$\text{Extract value (E.V)} = \frac{\text{weight of the extract (g)}}{\text{weight of powdered of leaf (g)}} \times 100$$

### Preliminary Phytochemical Screening of the Methanolic of Extracts

The preliminary phytochemical analyses of the extracts were carried out to determine the presence of carbohydrate, anthraquinones, tannins, saponins, alkaloids, and cardiac glycosides as described by [6, 7, 11].

**Test for carbohydrates****Molish's Test**

2 drops of molish's reagent was added to 2ml of the extract in a test tube and small quantity of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added down the side of the test tube. A reddish colour ring at the interface indicates the presence of carbohydrate.

**Fehling's Test**

5ml of an equal mixture of Fehling solution A and B was added and boiled on a water bath for 15minutes. Brick red precipitate indicates presence of reducing sugar.

**Test for Anthraquinones****Bonstrager's Test**

Small portion of the extract was soaked with 10ml of benzene and filtered. 5ml of 10% ammonia solution was added to the filtrate and stirred. The production of pink red or violet colour indicates the presence of free anthraquinones.

**Test for saponins****Frothing Test**

5 ml of the extract was vigorously shaken with 10 ml of distilled water in a test tube. Frothing which persisted was taken as an evidence for the presence of saponins.

**Test for Tannins**

Extract plus 4 ml of water and drops of ferric chloride were mixed. Immediate green precipitate was taken as evidence for the presence of tannins.

**Test for cardiac glycosides**

Test for Alkaloid 2 ml of the extract plus picric acid were mixed; an orange coloration was taken as evidence for the presence of alkaloids.

**Test for Alkaloid**

5ml of 20% HCl was used to dissolve little quantity of the extract. The solution was divided into three (3) portions.

**Meyer's Test**

2 drops of Meyers reagent was added to 1<sup>st</sup> portion. Formation of whitish precipitate indicates the presence of alkaloids.

**Wagner's Test**

2 drops of Wagner's reagent was added to 2<sup>nd</sup> portion of the extract. Formation of brown precipitate indicates the presence of alkaloids.

**Dragendoff's Test**

2 drops of Dragendoff's reagent was added to the 3<sup>rd</sup> portion of the extract. Formation of orange brown precipitate indicates the presence of alkaloids.

**Antimicrobial screening****Media preparation**

Nutrient agar was used as growth medium and was prepared according to the manufacturer's instruction (i.e. 28 grams in 2 liter of sterile distilled water). The prepared solution was sterilized at 121<sup>0</sup>C for 15minutes and was poured into sterilized petri-dishes. The plate were covered and left to solidify.

**Preparation of Different Concentration of the Extracts**

Various concentration of each extract were prepared by dissolving one gram of each extract in 10ml of sterile distilled water to obtain a stock solution of 200mg/ml. Appropriate serial dilution was then carried out to obtain concentration of 200mg/ml, 100mg/ml, 50mg/ml as well 25mg/ml for each extract.

### Screening of the Extracts for Antimicrobial Activity

The antimicrobial activities of the methanolic fresh leaf, shed leaf and root extract of *E. camaldulensis* were checked against the selected pathogenic microorganism in accordance with the agar well diffused method describe by [12]. The test organisms were standardized to 0.5 mcfarland standard to obtain bacteria density of  $10^6$  cfuml<sup>-1</sup>. Two hundred microliter of the standardized cell suspensions were spread on the nutrient agar (oxid). Four wells were bored using a sterile 7mm diameter cork borer and the wells were properly labeled.

The wells were filled with approximately 0.1ml of different concentration of the extract (200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml) and the fifth well was filled with 200mg/ml of control drug. The plates were allowed to stand on the bench at room temperature for about 2 hours for maximum diffusion of the extracts and the plates were then incubated at 37<sup>0</sup>C for 24 hours. After incubation, the zone of inhibition was measured using a transparent ruler. Duplicate of each plate were made to avoid contamination and very good ones were taken and recorded.

### Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the methanolic shed leaf, fresh leaf and root extract were determined using the method of [3]. Two fold dilutions of the methanolic leaves and root were carried out in nutrient broth. Duplicate tubes of each dilution were inoculated with  $10^6$  cfuml<sup>-1</sup> of the test organism and incubated at 37<sup>0</sup>C for 18 hours. After which they were examined for presence or absence of growth. The MIC was taken at the lowest concentration that represents the growth of the test microorganism.

### Determination of Minimum Bacteria Concentration (MBC)

The MBC of methanolic shed leaf, fresh leaf and root extract were determined by modification of the method of [22]. Samples were taken from plate with no visible growth in the MIC assay and subculture on freshly prepared nutrient agar plate and incubated at 37<sup>0</sup>C for 48 hours. The MBC was taken at the lowest concentration of the extract that did not show any growth on new set agar plate.

### Statistical Analysis

Diameters of zone of inhibition results from replicates were expressed as mean  $\pm$  standard deviation (SD). The data were analyzed by one-way Analysis of Variance (ANOVA), where significantly different, the mean values were separated using Duncan's Multiple Ranged Test (DMRT).

## RESULTS

### Phytochemical Screening

Qualitative phytochemical screening of the extract of fresh leaf and shed leaf of *Eucalyptus camaldulensis* indicated that both had carbohydrate, anthraquinones, triterpenes, saponins, cardiac glycosides, alkaloid and flavonoid (Table 1). However the root extract was found to contain only carbohydrate and other content tested were absent (Table 1)

**Table 1: Phytochemical Screening of the Methanolic Extract of Green Leaf, Shed Leaf and Root of *Eucalyptus camaldulensis***

S/N	Constituent	Test	Observation	Inference		
				Green Leaf	Shed Leaf	Root
1	Carbohydrate	Molish's test	Purple colour at the interface	+	+	+
		Fehling test	Brick red precipitate	+	+	+
2	Anthraquinones	Bonstrager's test	There is precipitation	+	+	-
3	Triterpenes	Leiberman-burehards test	There is colour change	+	+	-
4	Saponins	Frothing test	A honeycomb formed for more than 30mins	+	+	-
5	Cardiac glycosides	Kella-killiani test	Purple ring at the interface	+	+	-
6	Alkaloid	Mayer's test	There is precipitate	+	+	-
		Drangendoff test	There is precipitate	+	+	-
7	Flavonoid	Shinda's test	Red or orange colouration	+	+	-
		Ferric chloride test		+	+	-
		Sodium hydroxide test	Yellow colouration	+	+	-

Keys: + = Positive; - = Negative

**Table 2: Sensitivity Test and Zone of Inhibition of the Methanolic Green Leaf Extract of *Eucalyptus camaldulensis* and Ciprofloxacin (Control) against the Test Organisms**

Test Organism	Zone of Inhibition (mm)				
	Ciprofloxacin (mg/ml)	Methanolic Extract (mg/ml)			
	200	200	100	50	25
<i>Escherichia coli</i>	37.0±0.0 <sup>a</sup>	18.0±0.0 <sup>b</sup>	15.0±1.0 <sup>c</sup>	0.0±0.0 <sup>d</sup>	0.0±0.0 <sup>d</sup>
<i>Staphylococcus aureus</i>	38.0±0.0 <sup>a</sup>	24.0±2.0 <sup>b</sup>	21.5±2.5 <sup>b</sup>	18.0±2.0 <sup>b</sup>	17.0±3.0 <sup>b</sup>
<i>Salmonella typhi</i>	38.0±0.0 <sup>a</sup>	17.5±1.5 <sup>b</sup>	14.5±1.5 <sup>bc</sup>	13.0±0.0 <sup>c</sup>	0.0±0.0 <sup>d</sup>

**Table 3: Sensitivity Test and Zone of Inhibition of the Methanolic Shed Leaf Extract of *Eucalyptus camaldulensis* and Ciprofloxacin (Control) against the Test Organisms**

Test Organism	Zone of Inhibition (mm)				
	Ciprofloxacin (mg/ml)	Methanolic Extract (mg/ml)			
	200	200	100	50	25
<i>Escherichia coli</i>	37.0±0.0 <sup>a</sup>	17.5±0.5 <sup>b</sup>	14.0±0.0 <sup>c</sup>	0.0±0.0 <sup>d</sup>	0.0±0.0 <sup>d</sup>
<i>Staphylococcus aureus</i>	38.0±0.0 <sup>a</sup>	26.0±2.0 <sup>b</sup>	21.5±0.0 <sup>c</sup>	19.0±0.0 <sup>c</sup>	15.0±0.0 <sup>d</sup>
<i>Salmonella typhi</i>	38.0±0.0 <sup>a</sup>	16.5±0.5 <sup>b</sup>	14.0±0.0 <sup>bc</sup>	6.0±6.0 <sup>d</sup>	0.0±0.0 <sup>cd</sup>

**Table 4: Sensitivity Test and Zone of Inhibition of the Methanolic Root Extract of *Eucalyptus camaldulensis* and Ciprofloxacin (Control) against the Test Organisms**

Test Organism	Zone of Inhibition (mm)				
	Ciprofloxacin (mg/ml)	Methanolic Extract (mg/ml)			
	200	200	100	50	25
<i>Escherichia coli</i>	37.0±0.0 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>
<i>Staphylococcus aureus</i>	38.0±0.0 <sup>a</sup>	22.0±0.0 <sup>b</sup>	19.0±1.0 <sup>b</sup>	15.0±1 <sup>bc</sup>	7.0±7.0 <sup>c</sup>
<i>Salmonella typhi</i>	38.0±0.0 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>b</sup>

**Table 5: Comparison of Zone of Inhibition of the Methanolic green leaf, shed leaf and Root Extract of *Eucalyptus camaldulensis***

Test Organism	Zone of Inhibition (mm)		
	Green leaf	Shed Leaf	Root
<i>Escherichia coli</i>	8.25±3.15 <sup>a</sup>	7.88±3.01 <sup>a</sup>	0.00±0.0 <sup>b</sup>
<i>Staphylococcus aureus</i>	20.13±1.39 <sup>a</sup>	20.38±1.56 <sup>a</sup>	15.75±2.52 <sup>a</sup>
<i>Salmonella typhi</i>	11.25±2.56 <sup>a</sup>	9.13±2.72 <sup>a</sup>	0.00±0.0 <sup>b</sup>

Means ± standard error of means using analysis of variance, n=2 and superscript (a, b, c, d) indicating the order of mean along the rows. Means with the same superscript along the columns are not significantly different at P>0.05

**Table 6: The Minimum Inhibitory Concentration (MIC) of green leaf, shed leaf and root of *Eucalyptus camaldulensis***

Test organisms	MIC (mg/ml)		
	Green leaf	Shed leaf	Root
<i>Escherichia coli</i>	-	-	-
<i>Salmonella typhi</i>	50	50	-
<i>Staphylococcus aureus</i>	50	12.5	6.25

**Table 7: The Minimum Bactericidal Concentration (MBC) of green leaf, shed leaf and root of *Eucalyptus camaldulensis***

Test organisms	MBC (mg/ml)		
	Green leaf	Shed leaf	Root
<i>Escherichia coli</i>	-	-	-
<i>Salmonella typhi</i>	100	100	-
<i>Staphylococcus aureus</i>	50	50	25

## DISCUSSION

The result of the phytochemical screening of the methanolic fresh leaf and shed leaf extract of *Eucalyptus camaldulensis* revealed that, there was presence of anthraquinones, carbohydrate, cardiac glycoside, flavonoid, saponins, triterpenes. However, the root was found to contain only the carbohydrate. Previous research into the phytochemistry of the leaf of *Eucalyptus camaldulensis* revealed the presence of tannins, saponin and cardiac glycosides. [2 and 20] have independently reported the presence of these components in members of the family

Myrtaceae to which the plant used in this study belong. [5] reported that the phytochemical analysis of the crude extract of *Eucalyptus* species revealed the presence of saponin, saponin glycosides, steroids, cardiac glycosides, tannins, volatile oils, phenols and balsam gum. Thus, the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components. The antimicrobial activity could be partly explained by the presence of anthraquinones. The bacteriostatic and bactericidal activities of anthraquinone from *Cassia italic* have been established [15].

The result of the antimicrobial screening of the green and shed leaf extract of *Eucalyptus camaldulensis* was active against all the bacteria isolates used in this work. This is agreement with the work of [1] where it was discovered that at 10mg/ml, the extract of *Eucalyptus camaldulensis* was active against some bacteria isolates which include *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*.

The bacteria isolates used include two gram negative and one gram positive. The extract of both the green and shed leaf of *Eucalyptus camaldulensis* was active against gram negative and positive bacterial (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*), and root extract was active against only gram positive (*Staphylococcus aureus*). The inactivity of the root against gram negative bacteria may be due to absence of saponin and flavonoid in the root of the plant. This is because saponin and flavonoid are responsible for the antimicrobial and antifungal actions in plants [23].

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibiotic agent that can inhibit the growth of a microorganism and it is a measure of the type and amount of antibiotic that a patient will receive under treatment [4]. The Minimum Inhibitory Concentration (MIC) of the green leaf extract of *Eucalyptus camaldulensis* against the bacteria is 50mgml<sup>-1</sup>, shed leaf extract 12.5mgml<sup>-1</sup> and 50mgml<sup>-1</sup> and root extract 6.25mgml<sup>-1</sup>. The Minimum Bactericidal Concentration (MBC) of all the extract against the bacterial isolates range between 100 and 25mgml<sup>-1</sup>. However *Salmonella typhi* and *Staphylococcus aureus* show no activity for MBC against the root extract. The inhibitory effect of the extract of the three parts of plant against these pathogenic bacteria isolates can introduce the plant as potential candidates for drug development for the treatment ailment caused by these pathogens.

From the statistical analysis comparing the zone of inhibition of the fresh leaf, shed leaf and root extract with that of control antibiotics (Ciprofloxacin) using analysis of variance (ANOVA), it was discovered that there was significant difference between the control drug and the extracts. However, there were no significance difference between the antimicrobial activities of the green leaf and shed leaf but both showed significant inhibition as compared with the root of *Eucalyptus camaldulensis*.

### CONCLUSION

The green and shed leaves of *Eucalyptus camaldulensis* contain many phytochemical constituents while the root lack most of the phytochemical constituents.

The green and shed leaves extracts of *Eucalyptus camaldulensis* were active against gram negative and gram positive bacteria isolates used while the root was only active on gram positive bacterial.

Hence the extract can be used as basic ingredient for the development of drugs that will cure infectious diseases caused by such bacteria such as skin infections, typhoid fever, severe nausea, and some urinary tract infections.

### REFERENCES

- [1] B.A. Adeniyi and O. Ayepola, *Research Journal of Medicinal plant*, **2008**, 2, 34-38
- [2] Z. Ahmad, M. Mehomood and F. Mohamme, *Journal of Ethnopharmacology*, **1998**, 62, 183-193
- [3] D.A. Akinpelu and D.O. Kolawole, *Science Focus Journal*, **2004**, 7: 64-70.
- [4] J.M. Andrews, *Journal of Antimicrobial Chemotherapy*, **2001**, 48, 5-16.
- [5] H.I. Babayi, J.I. Kolo, P.O. Okogun, and U.J.J. Ijah, *Biokemistri*, **2004**, 16, 106-111.
- [6] P.A. Egwaikhde and C.E. Gimba, *Middle-East Journal of Scientific Research*, **2007**, 2, (3-4), 135-138.
- [7] W.C. Evans, In Trease and Evans Pharmacognosy, 14<sup>th</sup> edition, WB Saunders Company Limited, **1998**, pp. 15-16.
- [8] M.O. Fadeyi, A.O. Adeoye and I.D. Olowokudejo, *International Journal of Crude Drug Research*, **1987**, 27, 178-184.

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- [9] N.R. Farnsworth, O. Akerele, and A.S. Bingal, In Medicinal plant therapy, Bull WHO **1985**, pp. 631-981.
- [10] T. Ghosh, T.K. Mithy, P.K. Swain and A. Bose, *Pharmacognosy*, **2007**, 11(1), 204-208.
- [11] W.R. Gransden, S.J. Eykyn, I. Phillieps and B. Rowe, *Reviews of infectious Disease*, **1990**, 12, 1008-18.
- [12] J.B. Harborne, In *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 3<sup>rd</sup> edition, Chapman and Hall London, **1998**, pp. 1-198.
- [13] O.N. Irobi, M. Moo- young, W.A. Anderson and S.O. Daramola, *International Journal of Pharmacognosy*, **1994**, 34, 87-90.
- [14] R. Jeyachandran and A. Mahesh, *African Research Journal of Microbiology*, **2007**, 2(8), 645-649.
- [15] M.H. Kazmi, A. Malik, A. Hameed, N. Akhtar and S. Noor Ali, *Photochemistry*, **1994**, 36, 761-763.
- [16] J. Kerhorro, and J.G. Adam, *Plant medicine and toxicity*, Vigot Frered, **1973**, 579-580.
- [17] Mann, A.Y. Yahaya, A. Bansa and G.O. Ajayi, *African Research Journal of Microbiology*, **2008**, 2, 60-62
- [18] F.M. Pelezar, S.T. Chan, and L.I. Krieg, In *Microbiology*, 5<sup>th</sup> edition McGraw-Hill publishers Company limited, New York, **1993**, pp. 272-799.
- [19] L.M. Prescott, J.P. Harley and D.A. Klein, In *Microbiology*, 7<sup>th</sup> edition, McGraw- Hill publishers Incorporation New York, **2008**, Pp. 984-985.
- [20] Z.U. Sheriff, In *Modern Herbal Therapy for common Ailment*. Nature pharmacy series, spectrum Books Limited, Ibadan, Nigeria Association with Safari Books (Export) Limited, United Kingdom, **2001**, pp: 9-84.
- [21] Sofowora, In *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edition, John Willey and Sons limited, Ibadan, **1982**, 8-14.
- [22] A.L.R. Spencer, and J.F.T. Spencer, In *Public Health Microbiology, Method and Protocols*. Human press Incorporation, New Jersey, **2004**, Pp. 325-327.
- [23] G.E. Trease and W.C. Evans, In *text book of pharmacognosy*, Balliese Tindall London. **1998**, Pp. 156-158.
- [24] P.H. Warnke, S.T. Becker, R. Podschun, S. Sivananthan, I.N. Springer, P.A. Russo, J. Wiltfang, H. Fickenscher, and E. Sherry, *Journal Craniomaxillofac Surgery*, **2009**, 37(7), 392-397.