ISSN : 2249-5746

International Journal of Ayurvedic and Herbal Medicine 6:6 (2016) 2355–2363

Journal homepage: http://www.interscience.org.uk

Formulation Of A Herbal Dermal Ointment Using Whole Leaves Of *Cassia Alata* Linn.

Senga Kitumbe P[1], Nkanga Isalomboto C[2]*, Ngoro Vero[3], Welo Unya J[4], Opota Onya D[5]

[1,3,4,5]Faculty of Pharmaceutical Sciences, University of Kinshasa, P. O. Box 212 Kinshasa XI, Democratic Republic of the Congo.

[2]Chemistry Department, Faculty of Science, Rhodes University, P.O. Box 94, Grahamstown 6140, Eastern Cape, South Africa.

Corresponding author: E-mail address: stianolnkanga@gmail.com*

Abstract

The leaves of *Cassia alata* Linn. (*Fabaceae*) are famously used in Africa to treat dermatosis in traditional medicine. Many studies on *C. alata* have previously reported its antifungal and antibacterial activities that justify its medicinal uses. Traditionally, the leaves of *C. alata* are rubbed on affected body surfaces in order to treat skin infections. Drawing on this medicinal instruction, we have formulated a dermal ointment from the whole leaves of *Cassia alata* Linn. This was done in order to improve the traditional preparation used against dermal diseases. Some physicochemical characteristics of the formulation such as pH, phytochemical composition and chromatographic profile were studied. In comparison with the aqueous extract of *C. alata* leaves medicinal properties, gentamicin and ketoconazole discs were used as controls. The preliminary *in vitro* antibacterial and antifungal activities of the formulated herbal ointment have been evaluated against *Staphylococcus aureus* and *Candida albicans* strains. Overall, the herbal ointment demonstrated higher antifungal activity than antibacterial activity based on the zones of inhibition recorded for all the concentrations. The chromatographic fingerprints established and quantitative analyses conducted in this investigation are worth considering for the quality control of the herbal ointment formulated.

Key-words: *Cassia alata*, antifungal and antibacterial activities, ointment, dermatosis.

1. INTRODUCTION

The fast development of drug-resistant strains has attracted the attention of many researchers to exploit the biodiversity as a source for new anti-microbial agents.[1] Most of naturally occurring bioactive compounds that are currently being used as clinically appointed medicines are from plants. This makes medicinal plants to be a crucial source of therapeutic substances for the management of various human and animal diseases.[2]

In the field of infectious diseases, herbal-based medicinal preparations were the main basis of the medical remedy, before the establishment of antimicrobial drugs used nowadays .[3] With a growth of 12-15% per year, the need for herbal medicines is still rapidly growing despite the expansion of synthetic compounds in the world. In the last decade, the World Health Organization reported that the traditional use of medicinal plants to treat human diseases represented the primary health care strategy for 4 billion of the world population.[4]

Cassia alata is one of the plant species belonging to the family of *Fabaceae*. As a pan tropical ornamental shrub, averaging 2 – 3m of height, *C. alata* is largely distributed in tropical regions, expanding from Tropical America to India, Fiji, Indonesia, Malaysia and Africa. Also known as *Senna alata*, *Herpetic alata*

and *Cassia bracteata*,[5] *C. alata* is highly praised to be an effective medicinal weapon for the management of skin infections.[6] *C. alata* leaves are famously used in Africa especially to treat dermatosis in traditional medicine. Generally, the traditional procedure recommends rubbing vegetable materials on affected surfaces, but rarely aqueous extracts by decoction and infusion are also used.[7–10] Many recent studies have been conducted to provide scientific evidences regarding the efficacy of leaves of *C. alata* Linn and allowed justifying the medicinal potential of this plant. Evidences of antibacterial and antifungal activities of aqueous, methanolic, ethanolic and petroleum ether extracts of *C. alata* leaves against various bacteria and fungi species have been shown.[5, 11–18]

The preliminary phytochemical analysis of *Cassia alata* by *Idu et al.* revealed the presence of phenol, tannins, anthraquinones, saponins and flavonoids.[19] *Odunbaku* and *Lusanya* corroborated these findings and further stated that the plant also has got alkaloids and cardenolides.[20] The *C. alata* leaves have been qualitatively analyzed for the presence of anthraquinones compounds, namely rhein, aloe-emodin, chrysophanol, emodin, and physcion, as well as the flavonoid, kaempferol.[20,21] The antiseptic property of *Cassia alata*-based herbal soap against common pathogens of the skin has been studied and established.[13] *Alalor et al.* formulated antibacterial ointments with methanolic extract of *Cassia alata* leaves which demonstrated a considerable antibacterial activity towards common bacteria.[22]

The present study aimed to formulate an antibacterial and antifungal herbal ointment by using the whole *C. alata* leaves in order to take the advantage of all antimicrobial substances of this plant part in a single dosage form. The quality control and preliminary antimicrobial evaluations of the formulated herbal ointment have been conducted for characterization purpose.

2. MATERIAL AND METHODES

2.1. Plant Material

The fresh leaves of *C. alata* Linn, were collected from the plant on the main campus of the University of Kinshasa in March 2015. The identification and authentication of the plant were done by experts in the herbarium of the National Institute of Agronomic Research, University of Kinshasa/Democratic Republic of Congo.

2.2 Quality Control of Raw material

The leaves of *C. alata* Linn were dried at 40°C in oven for 48 hours, ground into powder and sieved using an electronic crusher (Thomas Scientific) with a 0.1mm metallic sieve.

2.2.1. Botanical Test

Identity and purity tests of raw material (*Cassia alata* L. leaves) have been performed by observing the organoleptic characters such as appearance, color and odor.

Phytochemical Screening

The detection of secondary metabolites of plant extract was carried out according to Kone et al. 23, Zellagui et al.24 and N'guessan et al.25 with slight modifications as described below. The analysis was focused on the dried methanolic extract of ground dried C. alata leaves. Briefly, the maceration of 20 g plant material was done in 125 ml of methanol 80° at room temperature for 48 hours. After filtration using cotton wool, the methanolic extract was dried at 40°C in the oven.

Identification of Alkaloids (By Draggendorf reagent)

A few drops of draggendorf reagent, a mixture of 0.85 g of basic bismuth nitrate, 10 ml of glacial acetic

acid and 40 ml of distilled water; were introduced into a test tube containing 2 ml of the methanolic extract solution. The formation of an orange-red precipitate should have indicated the presence of alkaloids.[23]

Identification of anthracenosides (reaction of Borntraeger)

An aliquot of extract was dissolved in 5 ml of HCl diluted to 1/5 and heated in a boiling water bath for 30 minutes, and then extracted with 20 ml of $CHCl_3$ after cooling. 1 ml of 25% of NH_4OH was added to organic phase and the mixture was shaken for a short while. The appearance of a color ranging from red to purple indicated a positive reaction.[24, 25]

Identification of Sterols and Polyterpenes (reaction of Liebermann)

The extract was dissolved in 0.5 ml of acetic anhydride and 0.5 ml of $CHCl_3$ was added. Then 1 ml of concentrated sulfuric acid was carefully and slowly added. At the contact zone of two liquids, the appearance of a brownish red ring should have indicated the presence of sterols and polyterpenes.[24]

Identification of Tannins (ferric chloride 1% reaction)

To 1 ml of aqueous solution of extract, 3 drops of ferric chloride were added. The appearance of blackish blue color indicated the presence of gallic tannins, while the green or dark green color should have indicated the presence of catechin tannins.[23, 24]

Identification of Flavonoids (reaction of Shinoda)

To 2 ml of methanolic extract solution, 5 drops of concentrated HCl and metallic magnesium were added. The appearance of red or orange color should have indicated the presence of flavonoids.[24, 25]

Identification of Anthocyanosides

The alcoholic solution of the extract was acidified. The acidic solution should have become red at pH=7 and not changed to green or violet at alkaline medium in the presence of anthocyanosides.[24]

Identification of Saponins (foam index)

0.1 g of the extract was introduced in a test tube containing 10 ml of distilled water for dissolution. The tube was shaken vigorously for 45 seconds in the lengthwise and then allowed to stand for 15 minutes, and then the foam height was measured. The persistence of foam more than 1 cm indicated the presence of saponins.[25]

2.2.2. Assay of Anthraquinone Aglycones

1 g of dried powdered *C. alata* leaves was boiled in 110 ml of distilled water for 5 minutes. To 50 ml of this aqueous extract, 5 drops of concentrated sulfuric acid were added, and the mixture was boiled for 5 minutes. After cooling, anthraquinone aglycones have been extracted by chloroform (3 x 25 ml) and the organic extract was evaporated to dryness in a tarred flask to yield 0.02 g anthraquinone aglycones for 50 ml of aqueous extract, or 0.044 g for 110 ml. The Borntraeger's reaction was carried out on the aliquot of the residue.[26, 27]

2.3. Preparation of the Ointment

The strategy of formulation adopted in this study consisted of incorporating plant material in topical ointment bases. In this method, the constituents of the base (53.2 g of Vaseline and 26.6 g of lanolin) were placed together in a melting pan and allowed to melt together at 70°C. On the other hand, 10 g of dried

powdered plant material were mixed up with 10 g of vegetable oil in a ceramic mortar with a pestle. The ointment preparation was performed by incorporating and triturating the fluid topical vehicle prepared in the mixture above to obtain 100 g of herbal ointments containing 10 % w/w of *C. alata* leaves. After adding 0.2 g of menthol as an additive agent, the prepared herbal ointment was put in jars, labeled and stored at room temperature.

2.4. Quality Control of The Formulated Ointment

Organoleptic Characters

The organoleptic characteristics of the formulated ointment were established by assessing its appearance, odour, texture and colour.

Homogeneity

The ointment formulated was spread out over a flat surface in a thin layer, using a spatula, on the purpose of assessing its homogeneity.

Determination of the pH

The pH of the formulated ointment was determined by using Digital pH meter. 5 g of the weighed formulation was dispersed in 50 ml of hot distilled water and the pH was measured. And by comparison, the pH of the decoction of the plant material was determined in the same conditions, by using 0.5 g of dried powdered of *C. alata* leaves in 50 ml of distilled water.[22]

Determination of anthraquinone aglycone content

10 g of the formulated ointment was boiled in 110 ml of distilled water for 10 minutes. To 100 ml of the decanted aqueous phase, 10 ml of concentrated sulfuric acid were added, and the mixture was boiled for 5 minutes. After cooling, anthraquinone aglycones were extracted by the chloroform (3 x 50 ml), and the organic extract was evaporated to dryness in a tarred flask yielding 0.04 g anthraquinone aglycones for 100 ml of the aqueous ointment extract, or 0.044 g for 110 ml. The Borntraeger's reaction was carried on the aliquot of the residue.[26, 27]

Thin Layer Chromatography

The residue of anthraquinone aglycone obtained above was dissolved in 5 ml of ethanol and the obtained solution (spot ISA) was chromatographed using anthraquinone aglycones extracted (at 2.2.3) from plant material (spot CAL) as reference.

TLC Aluminium pre–coated plates with Silica gel60 GF254 (10x5 cm; 0.2 mm thick, Merck) were used with two different mobile phases (Vigor et al., 2010). The twin trough glass chamber was saturated for 30 minutes with ethyl acetate – methanol – water mixture (7.7 :1.3 :1 v/v) as the first mobile phase. TLC plates were developed to 3.75 cm distance above the position of the sample application. The plates were removed from the chamber and air dried at room temperature.

For the second development, Toluene - Ethyl Formiate - HCOOH (7.5 :2.4 :0.1) was used as mobile phase. Another twin trough glass chamber was saturated with this second mobile phase for 30 minutes. And the dried TLC plate was developed again to 7.5 cm distance above the position of the sample application. The revelation has been done by the visual observation of TLC plates before and after the exposition to the ammoniac vapors.[27]

2.5. Antimicrobial Evaluations

The *Staphylococcus aureus* and *Candida albicans* strains ready for testing were obtained respectively from the culture collection of the National Institute of Biomedical Researches (INRB) and the Microbiology Research Laboratory of the Faculty of Pharmaceutical Sciences/University of Kinshasa; both located in Kinshasa, Democratic Republic of Congo. These two pathogens were selected as bacterial and fungal model species due to the fact that they are part of the main microorganisms involved in polymicrobial infections such as topical conditions.[28, 29] The microbial cultures were streaked in appropriate culture media, Chapman and chloramphenicol-based Sabouraud media namely.

To prepare herbal ointment samples for antimicrobial assessment, 100 g of the ointment were boiled for 5 minutes in 500 ml of distilled water and the aqueous phase was decanted off to provide a stock solution from which various suitable concentrations of aqueous extract were prepared, 100mg/ml; 150 mg/ml and 200mg/ml.[11, 14, 30] On the other side, 100 g of blank ointment and 10 g dry powder of *C. alata* leaves were treated in the same conditions to produce respectively negative and positive control extracts at the same concentrations. Gentamicin- and ketoconazole-based discs were used as references to confirm drug-sensibility of the microbial species used in this study.

The antimicrobial susceptibility testing was conducted by disc diffusion method as described by *Zaidan et al.*[31] Sterile testing discs of 5 mm have been completely soaked in sample solutions as well as for the controls, and allowed to dry at 37°C and placed into Mueller-Hinton Agar media containing single colonies of either *Staphylococcus aureus* or *Candida albicans*, Gentamicin- or Ketoconazole-based reference discs also were introduced accordingly. The Petri dishes were allowed to incubate at 37°C for 24 hours and the zones of inhibition were measured in mm diameters to estimate antimicrobial activities.

3. RESULTS AND DISCUSSION

3.1. Plant Material

Botanical tests

The harvested plant *C. alata* Linn. (*Fabaceae*) were identified by experts in the herbarium of the National Institute of Agronomic Research, University of Kinshasa, Democratic Republic of Congo. The dried powder from *C. alata* leaves is brownish, scented and slightly prickly.

Phytochemical Screening

The results of characterization tests of secondary metabolites carried out on the methanolic extract of dried powdered *C. alata* leaves indicate the presence of Saponins, Gallic tannins and Anthracenosides ; as described in the Table 1 below.

Table 1: Phytochemical screening of methanolic extract *C. alata* leaves

Secondary metabolites	Reagents	RESULTS
Saponins	Foam index	+
Sterols & polyterpenes	Liebermann	-
Gallic tannins	$FeCl_3$ 1%	++
Flavonoids	Shinoda	-
Catechic tannins	$FeCl_3$ 1%	-
Anthocyanosides	H_2SO_4 & NH4OH	-
Anthracenosides	Borntraeger	+++
Alkaloids	Dragendorff	-

- : Absence; + : Presence ; ++ : Abundant ; +++ : very abundant.

3.2. Formulated Ointment

Organoleptic Characters

The formulated ointment presented most of organoleptic characters of the plant material. The ointment was brownish, menthol scented and slightly prickly; as pharmaceutical ingredients used were neutral.

Homogeneity

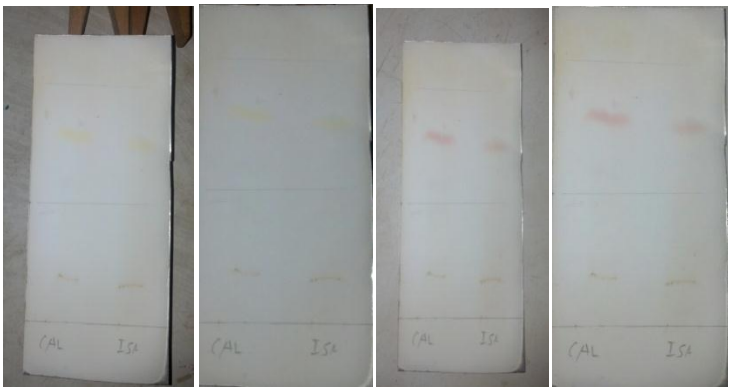
The homogeneity test showed that the formulated ointment, spread out over a flat surface in a thin layer, was homogeneous.

Determination of the pH

The pH of formulated ointment (5.6) was close to the plant material decoction's one (5.56). This pH is considered acceptable to avoid the risk of skin irritation upon application.[22]

Thin Layer Chromatography

Chromatographed side by side, anthraquinone aglycones from plant materiel (CAL) and from formulated ointment gave both two visibly stains before the revelation, the lowest compound was brownish with R_f = 0.11 and the highest was yellow with R_f = 0.52; and after the exposition to the ammoniac vapor, the lowest stain color did not change (brownish) but the highest stain became red (Borntraeger's reaction), as Figure 1 below shows.



A B C D

Figure 1: TLC fingerprints of the formulated ointment (ISA) with raw material (CAL, *C. alata* leaves); A – B : Plates without the ammoniac vapors; C - D: Plates with the ammoniac vapors.

Anthraquinone Aglycone Content

The results of anthraquinone agycone content determination show that the dried powdered leaves of *C. alata*, used as raw material, contain 4.4 % of the anthraquinone aglycone ; but the formulated ointment contains 0.44 % of the anthraquinone aglycone, as described in Table 2.

Table 2: Results of the anthraquinone aglycone assay

Samples	Aliquot (g)	Aglycone (g)	Content (%)
Plant Material	1	0.044	4.4
Formulated ointment	10	0.044	0.44

3.3. Antimicrobial Evaluations

The results of antimicrobial assessment are presented in Table 3 below as follows.

Table 3: Diameters (mm) of zones of inhibition from samples and references evaluated

Microorganisms	Inhibition Zone (mm)				
	Herbal Ointment (mg/ml) 100 150 200	*C. alata* leaves (mg/ml) 100 150 200	Blank Ointment (mg/ml) 100 150 200	Gentamicine 10 µg	Ketoconazole 10µg
S. aureus	6 8 13	8 10 15	- -	25	-
C. albicans	9 12 16	11 15 19	- -	-	19

The above inhibition zone values presented in the Table 3 above confirm antifungal and antibacterial properties of *C. alata* leaves previously reported by many research groups. The present findings also show that both the prepared herbal ointment and the crude dry *C. alata* leaves showed highest antimicrobial activities at the concentration of 200 mg/ml of the extract as compared to other concentrations. All the evaluated extracts showed more antifungal activity than antibacterial activity irrespective of the concentration. Only the blank ointment did not show any antimicrobial activities, a fact that proves the efficacy of the prepared herbal based ointment. Gentamicin-based 10µg discs used in this study as reference showed higher antibacterial activity than the crude *C. alata* leaves extract, 25 mm zones of inhibition versus 15 mm; while the antifungal activity of Ketoconazole-based 10µg disc was equal to that of the plant extract, 19 mm zone of inhibition for both. This observation supports the famous antifungal stutas of *C. alata* leaves, also reported by many other studies.[5, 11–19] The antimicrobial properties of the non formulated *C. alata* leaves were higher than the formulated herbal ointment, with respectively 15 mm versus 13 mm zones of inhibition for antibacterial evaluation; and 19 mm versus 16 mm for antifungal assessment. Such antimicrobial properties had been previously classified in the same category by *Makinde et al.*,[14] zones of inhibition being in the rang of 10 – 20 mm.

4. CONCLUSION

The aim of the present research was to formulate a herbal ointment from the whole leaves of *C. alata* Linn. The findings obtained in this investigation confirmed antimicrobial activities of *C. alata* Linn leaves previously reported, antifungal effect being more pronounced than the antibacterial one. The formulated *C. alata* Linn leaves-based ointment also showed almost the same antimicrobial profile, though lesser than that of pure leaves extract. The quality control study conducted would be useful for the qualitative and quantitative analyses of the formulated herbal ointment. This study would encourage further investigations on *C. alata* leaves-based herbal ointments, and initiate the manufacture of a pilot share of the prepared topical formulation for clinical trials.

5. REFERENCES

1. Latha PS, Kannabiran K. Antimicrobial activity and phytochemicals of Solanum trilobatum Linn. Afr J Biotech 2006; 5 (23): 2402 –2404.
2. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected indian medicinal flora. Turkish Journal of Biology 2005; 29: 41–47.
3. Akinyemi KO, Coker AO, Bayagbon C, Oyefolu AOB, Akinside KA and Omonigbehin EO.

Antibacterial screening of five Nigerian Medicinal Plants Against *S.typhi* and *S. paratyphi*. Journal of the Nigeria Infection Control Association 2000; 3:1-4.

4. Kalia AN. Textbook of Industrial Pharmacognosy. 1st edition 2000, CBS publishers and distributors 9.
5. Abubacker MN, Ramanathan R, Senthil Kumar T. *In vitro* antifungal activities of *Cassia alata* Linn. Flower extract. Natural product Radiance 2008, 7(I): 6-9.
6. Igoli JO, Ogaji OG, Igoli NP, Tor-Anyiin TA. Traditional medicinal practices among the Igede people of Nigeria (part II). Afri. J. Tradit. Compliment Altern. Med. 2005; 2(2): 134-152
7. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, Etoa FX, Ngongang J. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.) Roxb (Ceasalpiniaceae). African Journal of Biotechnology 2006; 5 (3): 283 – 289.
8. Chidumé FC, Gamaniel K, Amos S, Akah P, Obodozié O, Wambebe C. Pharmacological activity of the méthanolic extract of *Cassia nigricans* leaves. Indian Journal of Pharmacology 2001 ; 33: 350-356 .
9. Abatan MO. A noteon the anti-inflammatory action of plants of some *Cassia*. Fitoterapia 1990; 61 (4) : 336 - 338.
10. Palanichamy S, Nagarajan S. Antifungal activity of *Cassia alata* leaf extract. J Ethnopharmacol. 1990; 29: 337 – 340.
11. Alalor CA, Igwilo CI, Jeroh E. Evaluation of the antibacterial properties of aqueous and methanol extracts of *Cassia alata.* J. Pharm. and Allied Health Sci. 2012; 2 (2): 40-46.
12. Timothy SY, Wazis CH, Adati RG, Maspalma ID. Antifungal Activity of Aqueous and Ethanolic Leaf Extracts of *Cassia Alata* Linn. Journal of Applied Pharmaceutical Sciences 2012 ; 2 (7) : 182 – 185.
13. Esimone CO, Nworu CS, Ekong US, Okereke, B. Evaluation of the antiseptic properties of Cassia alata-based herbal soap. Internet J Altern Med. 2008; 6(1). DOI: 10.5580/b0e
14. Makinde AA, Igoli JO, TA'Ama L, Shaibu SJ, Garba A. Antimicrobial activity of *Cassia alata.* African Journal of Biotechnology 2007; 6 (13) : 1509-1510.
15. Owoyale JA, Olatunji GA and Oguntoye SO.Antifungal and antibacterial activities of an alcoholic extract of *Senna alata* leaves. Journal of Applied sciences & Environmental Management 2005; 9 (3): 105 – 107.
16. Reezal I, Somchit MN and Abdul Rahim M. *In Vitro* Antifungal Properties of *Cassia alata* (Gelenggang Besar). Proceedings of the Regional Symposium on Environment and Natural Resources 10-11th April 2002, Hotel Renaissance Kuala Lumpur, Malaysia 2002; 1 (13): 654 – 659.
17. Adedayo O, Anderson WA, Moo-young M, Suceckus V, Patil PA and Kolawole DO. Phytochemistry and antibacterial activity of *Senna alata* flower. Pharmaceutical. Biology 2001; 39 (6): 408 – 412.
18. Khan MR, Kihara M, Omolosa AD. () Antimicrobial Activity of *Cassia alata*, Fitoterapia 2001; 72, 561 – 564.
19. Idu M, Omonigho SE, Igeleke CL. Preliminary investigation on the Phytochemistry and antimicrobial activity of *Senna alata* L. Flower. Pak J Biol Sci. 2007; 10: 806 – 809.
20. Odunbaku OA, Lusanya OAF. Synergistic effect of ethanol leaf extract of *Senna alata* and antimicrobial drugs on some pathogenic microbes. Adv Environ Biol. 2011; 5: 2162 – 2165.
21. El-Mahmood AM, Doughari JH. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. Afri J Pharm Pharmacol. 2008; 2 (7): 124 – 129.
22. Alalor CA, Igwilo CI, Azubuike CP. Evaluation of the Antibacterial activity of Herbal ointments

formulated with Methanolic extract of *Cassia alata.* Asian Journal of Biomedical and Pharmaceutical Sciences 2012; 2(13) : 15-19.

23. Kone M, Ouattara K, Gnahoue G, Ouattara A, Coulibaly A. Study Ethnopharmacological and Phytochemical Screening of Some Plants Involved in the Treatment of Abdominal Infections in The Department of Kouto (Cote d'Ivoire). Sch. J. App. Med. Sci. 2013; 1(2):56 – 61.
24. Zellagui A, Derouiche K, Gherraf N, Rhouati S. Characterisation of Secondary Metabolites and Evaluation of Antibacterial activity of two Algerian species: *Launaea glomerata* (Cass. Hook. F. *and Cynara cardinculus* var. *silvestris* (Lamk.). J. Microbiol. Biotech. Res. 2012 ; 2 (5): 736 – 740.
25. N'guessan K, Kadja B, Zirihi N, Traoré D, Aké-assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire) Sci. Nat. 2009 ; 6 (1) : 1 – 15.
26. Vigor C, Vercauteren J, Montels J. Travaux Pratiques de Pharmacognosie (3ème année) : Les substances naturelles dans la chaine du médicament. Université de Montpellier I, Laboratoire de Pharmacognosie 2010, 48p.
27. British Pharmacopoeia. Herbal Drugs and Herbal Drug Preparation *Senna* leaves ; London 2009, Vol III.
28. Brian M Peters, Raven M Ward, Hallie S Rane, Samuel A Lee, Mairi C Noverr. Efficacy of Ethanol against Candida albicans and Staphylococcus aureus Polymicrobial Biofilms. Antimicrob. Agents Chemother. 2013; 57 (1): 74 – 82.
29. Alsterholm, Mikael; Karami, Nahid; Faergemann, Jan . Antimicrobial activity of topical skin pharmaceuticals - an in vitro study. Acta Derm Venereol. 2010; 90 (3): 239 – 245.
30. Ali-Emmanuel N, Moudachirou M, Akakpo AJ, Quetin-leclercq J. Activités antibactériennes *in vivo* de cassia alata, lantana Camara et mitracarpusscaber sur dermatophilus congolensis isolé au Benin. Revue Elev. Méd. Vét. Pays Trop. 2002 ; 55(3):183-187.
31. Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine 2005; 22(2): 165–170