ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Preliminary phytochemical analysis and a comparative study of the antibactrial activity of *Cyndon dactylon (L) Pers* roots.

Abdelaali Atmani, Lakhdar Sekhri*, Bilal Khaled, and Ahmed Tabchouche.

Laboratoire de Dynamique Interaction et Réactivité des Systèmes, Process Engineering Department, Faculty of Applied Sciences, University Kasdi Merbah, Ouargla 30000, Algeria.

ABSTRACT

The present work is aimed mainly to investigate and compare the antibacterial activities of some extracts of the of *Cyndon dactylon (L) Pers* roots against six bacteria strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Staphylococcus Coagulasse* (ATTC 5118), *Staphylococcus aureus* ATCC 25923, *Klebcsiella pneumonie*, and *Enterococcus faecalis* using Disc diffusion method. The results revealed that all extracts exhibited a certain bioactivity against all tested gram positive and gram negative bacteria at 1000 and 5000 µg/ml. Moreover the Ethanol/H_2O extracts showed higher activity compared to ethyl acetate, dichloromethane and n-butanol extracts, where the maximum activity was recorded against *Staphylococcus aureus* and its maximum inhibition diameter of 16 mm at concentration 5000 µg/ml, whereas the ethyl acetate and the dichloromethane extracts showed no effect against *Escherichia coli, Pseudomonas aeruginosa*, *Klebcsiella pneumonie, Enterococcus faecalis*) at 500 µg/ml. The results obtained in the present study suggest that the *Cyndon dactylon* can be used in treating diseases caused by the tested organisms. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plants responsible for the antimicrobial activity.

Keywords: *Cyndon dactylon,* phytochemical, Staphylococcus aureus, ethanolic extract.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Medicinal plants are rich in biological active compounds and it holds healthier and harmless alternate to synthetic drugs. Extracts of the medicinal plants are useful in the treatment of several health problems; bacterial infections such as the urinary tract infection, that is the most common bacterial diseases in children, as it ranks second in terms of spreading infection after respiratory tract [1-4]. The urinary tract infection comes usually from attacking microorganisms urinary system that are mostly negative gram bacteria, from digestive system, as most of the infections at urinary system caused by bacteria intestinal Enterobacteriaceae including *Bacillus* colon *Escherichia coli*, which occupies a leading position among the races of this family [5] as well as other pathogens include *Staphylococcus aureus* and *Streptococci*.

Therefore, we have chosen the study roots of medicinal *Cyndon dactylon (L) Pers* plant, belongs to family of Poaceae, because its extracts is useful in the treatment of several health problems and has several significant applications such as [an anticancer, anti-diuretic, anti-inflammatory] [6], anti-diabetic [6-7], hypolipidemic [8], healing of minor injuries [9], anti-emetic, purifying agent and dysentery [10]. In a recent study, the extracts of *C. dactylon* had also been reported to be effective for antimicrobial activity against bacterial pathogens and fungus [11]. Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity [12-14]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [15-20].

The aim of of this study was to evaluate the activity of EtOH/H_2O, n-butanlic alcohol, dichloromethane, and ethyl acetate extracts against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

Since the *Cyndon dactylon (L) Pers* is used in local folk medicine to cure some diseases such as urinary tract infection, we wish to report the study and evaluation of the activity of EtOH/H_2O, n-butanlic alcohol, dichloromethane, and ethyl acetate extracts against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

Cyndon dactylon (L) Pers is known locally as "Nadjem or Affar"

MATERIALS AND METHODS

Fresh *Cynodon dactylon (L) Pers* plant was collected from the mountains of Arris-Batna-Algeria, in November 2015. The plants were deposited at Lab. Dynamic de Interaction et Réactivité des Systèmes, Department of Process engineering, Faculty of Applied Sciences, University of Kasdi Merbah-Ouargla, Algeria. Fresh roots material was washed under running tap water, air dried under dark and then homogenized to fine powder and stored in closed container away from light and moisture.

Preliminary Phytochemical Analysis

Qualitative Phytochemical analysis of the plant powder was determined as follows:

Resins : (10 ml plant material in 20 ml distilled water, filtered) ; a 10 ml filtrate + 4% HCl, the appearance of turbidity indicated the presence of *Resins* [21]; ***Volatile oils*** (10 ml plant material in 10 ml distilled water, filtered), the filter paper was then impregnated with the filtrate and exposed to the UV rays, bright rose color indicated the presence of *Volatile oils* [22]; ***Coumarins***: In a test tube was placed 1g of plant material in 10 ml of distilled water, and then covered with filter paper after being soaked in a diluted solution of NaOH. The test tube was placed in boil water bath for few minutes and then exposed to a source of UV rays, yellow-green indicated the presence of *Coumarins* [23]; ***Terpenes*** and ***steroids*** (Liebermann-Burchard reaction) : 1mg plant material in 10 ml chloroform, filtered) ; a drop of acetic anhydride + a drop conc. H_2SO_4. The brown color indicated the presence of *Terpenes*. If the mixture left for few minutes; the appearance of blue color indicated the presence of *steroids* [24]; ***Phenols*** **:** (200 mg plant material in 10 ml distilled water, filtered) ; a 2 ml filtrate

+ 2 ml $FeCl_3$, blue-green precipitate indicated the presence of *Phenols* [24]; ***Tannins***: (10 g plant material in 50 ml distilled water, filtered); a 2 ml filtrate + 2 ml of 1% $FeCl_3$, blue-black precipitate indicated the presence of *Tannins*; ***Alkaloids*****:** (200 mg plant material in 10 ml methanol, filtered) ; a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayer's reagents/Wagner's reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of alkaloids[25]; ***Saponins***: method 1: (1g of plant material in 10 ml distilled water was placed in the test tube and shaked strongly); frothing persistence indicated the presence of saponins. Method 2: (1 to 3 ml of sol. 1% $HgCl_2$ was added to 5g of plant material; the appearance of white precipitate indicated the presence of *saponins*); ***Glycosides*** (Keller-Kilani test: a 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of Glycosides; ***Steroids*** (Liebermann-Burchard reaction: 200 mg plant material in 10 ml $CHCl_3$, filtered); a 2 ml acetic anhydride + conc. H_2SO_4. Blue-green color indicated the presence of steroids; ***Flavonoids***: (200 mg plant material in 10 ml ethanol, filtered) ; a 2 ml filtrate + conc. HCl + magnesium ribbon, pink-tomato red color indicated the presence of flavonoids.[25] ***Flavons***: 10 ml of solution of plant powder in ethanol (50%) was added to 10 ml of KOH solution (50%), and then equal amounts of this solution and extracted plant were mixed, yellow color, indicated the presence of *Flavons* [26]. indicates the absence of phyto-constituent. Moreover, the results of this preliminary phytochemical analysis of *Cyndon dactylon (L) Pers* roots indicate the absence of Volatile Oils.

Extraction of plant material

The extracts were prepared by soaking 200 g of the roots powder in petroleum ether for 24 hours in order to get rid of the fat and chlorophyll. The mixture was then filtered and the residue soaked again in a mixture of EtOH/water (70/30) for 24 hours with shaking from time to time and then filtered. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The filtrates were subjected to extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). The organic phases were separated and evaporated. The resulting residues were stored at 4°C.

Microorganisms

All bacterial standard strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Staphylococcus Coagulasse* (ATTC 5118), *Staphylococcus aureus* ATCC 25923, *Klebcsiella pneumonie*, *and Enterococcus faecalis* were obtained and diagnosed in Microbiology Laboratory, Arris-Batna Hospital, Algeria.

Preparation of the bacterial culture media

3.7 of muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm for 15 min. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 5 mm.

Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [26].

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of anti-bacterial activities of plant extracts. A bacterial suspension adjusted to 0.5 Mc Farland standard ($1.5x10^8$ CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri discs and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter. The resulting

ISSN: 0975-8585

residue of all extracts stored at 4°C were tested at concentrations of 500, 700, 1000 and 5000 µg/ml and were prepared in DMSO.

RESULTS

The preliminary phytochemical analysis of the crude roots powder of *Cyndon dactylon (L) Pers* plant collected showed that this plant contains many active ingredients: *Coumarins, tannins, volatile oils, terpenes and alkaloids*, one of the antioxidants of the bacteria responsible for the effect of microbs, also contains flavonoids including glycosides antioxidant and phenols and saponins.

Results for antibacterial activity as obtained with *Cyndon dactylon (L) Pers* plant revealed that the four different extracts tested in vitro by agar disc difusion against six bacterial species. **Table 1** : summarizes the microbial growth inhibition of tested extracts of this plant that showed significant bacterial activity against all the tested bacteria (*Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus Coagulasse*, *Staphylococcus aureus*, *Klebcsiella pneumonie, Enterococcus faecalis*), where the maximum activity was recorded against *Staphylococcus aureus* and a maximum inhibition diameter of 16 mm with the EtOH/water extract at concentration 5000 µg/ml ; whereas the ethyl acetate extract showed no effect against *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus Coagulasse*, *Klebcsiella pneumonie, Enterococcus faecalis*) at 500 µg/ml

Table 1: Antibacterial activity of extracts of *Cyndon dactylon (L) Pers* roots

		(Diameter of inhibition zone (mm)					
Plant extracts (µg/ml) \ **Bacteria strains**		**Escherichia coli (TTC25922)**	**Pseudomonas aeruginosa (TTC 27853)**	**Staphylococcus aureus (TTC 25293)**	**Staphylococcus coagulasse (TTC 5118)**	**Klepsiella pneumonie**	**Entéro coque faecale**
Dichloromethane extract	500	-	-	**07**	**06**	-	-
	700	07	-	**07**	**07**	-	06
	1000	**08**	**08**	**10**	**10**	**06**	**07**
	5000	**08**	**08**	**11**	**10**	**06**	**07**
Ethyl acetate extract	500	**-**	**-**	**07**	-	-	-
	700	**08**	**-**	**08**	**06**	-	**06**
	1000	**10**	**06**	**10**	**08**	**09**	**08**
	5000	**10**	**07**	**10**	**08**	**09**	**08**
Butanol extract	500	**06**	-	06	-	-	-
	700	**07**	**09**	**09**	**08**	**-**	**07**
	1000	**09**	**10**	**13**	**10**	**07**	**07**
	5000	**09**	**10**	**13**	**11**	**07**	**08**
EtOH/H_2O	500	-	-	-	-	-	-
	700	-	-	-	-	-	-
	1000	**11**	**07**	**14**	**12**	**09**	**08**
	5000	**11**	**07**	**16**	**12**	**10**	**08**

Moreover the dichloromethane extract showed no effect against *Escherichia coli, Pseudomonas aeruginosa*, *Klebcsiella pneumonie* and *Enterococcus faecalis*). Moderate inhibition was recorded with ethyl acetate extract at concentrations 1000 and 5000 µg/ml against all the bacteria tested. As far as the Ethanol/water extract is concerned a significant bacterial activity against all the bacteria was recorded at the concentrations 1000 and 5000 µg/ml. On the other hand Ethanol/water extracts were ineffective against *all bacteria* at concentration 500 and 700 µg/ml. **Figures-1, 2, 3, and 4** showed the influence of the extract concentration four the different extracts on the growth of the tested bacteria.

ISSN: 0975-8585

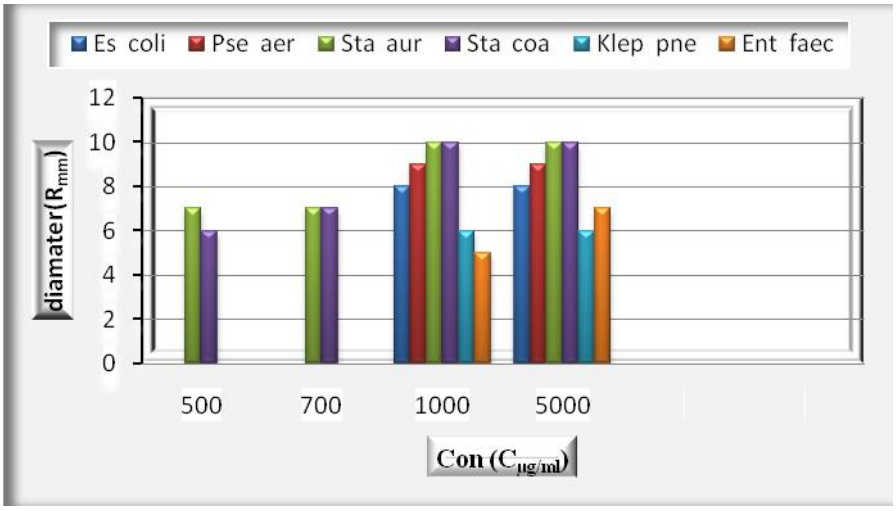

Figure 1: The influence of three different extracts concentrations of dichloromethane (µg/ml) of *Cyndon dactylon (L) Pers* plant *vs* the inhibition diameter (mm) on the tested bacteria

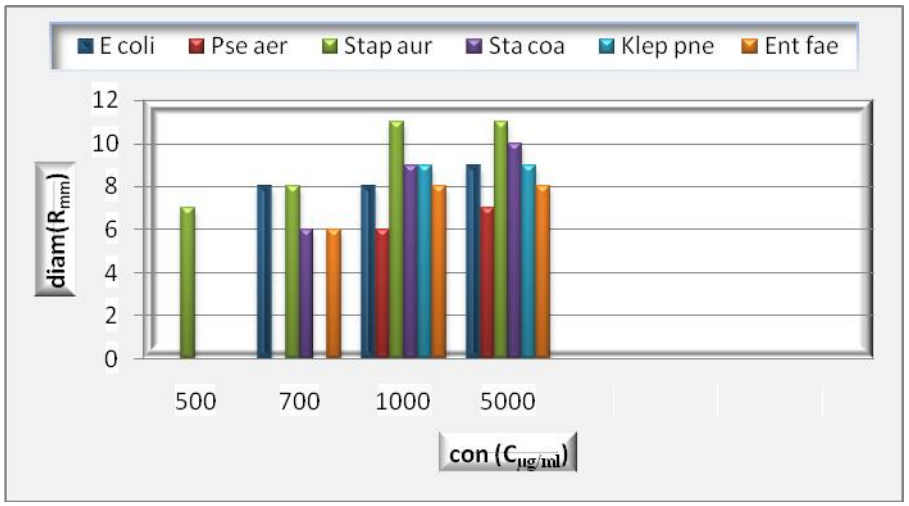

Figure 2: The influence of three different extract concentrations of ethyl acetate (µg/ml) of *Cyndon dactylon (L) Pers* plant *vs* the inhibition diameter (mm) on the tested bacteria

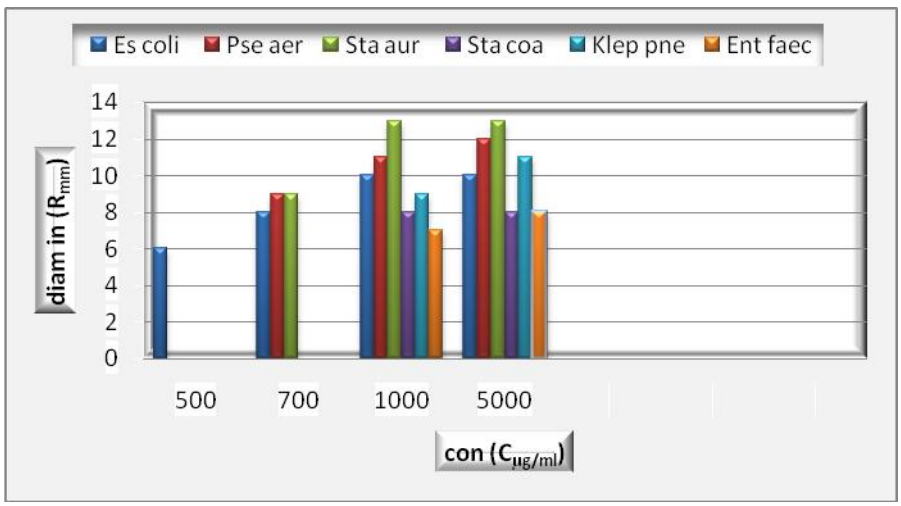

Figure 3: The influence of three different extract concentrations of butanol (µg/ml) of *Cyndon dactylon (L) Pers* plant *vs* the inhibition diameter (mm) on the tested bacteria

ISSN: 0975-8585

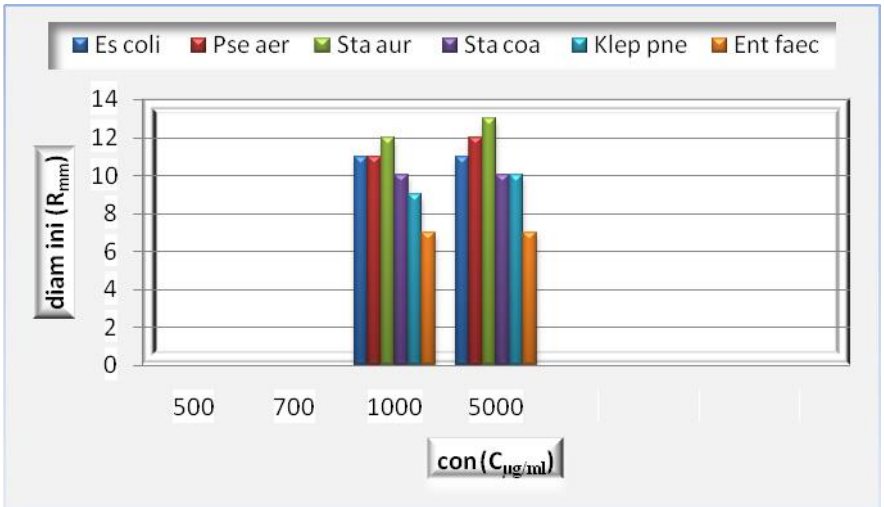

Figure 4: The influence of three different extract concentrations of EtOH/H_2O (µg/ml) of *Cyndon dactylon (L) Pers* plant *vs* the inhibition diameter (mm) on the tested bacteria

DISCUSSION

The increase in the effect of the alcoholic extracts of some plants may be due to the extract effect on the permeability of the cell membrane and the function of the bacterial cell Al-Abed, Kaouther Fouad [28]. Since the alcoholic extracts (EtOH and n-BuOH) are more polar than dichloromethane and ethyl acetate extracts, so it has the ability to extract the largest quantities of the active substances such as phenols flavonoids [Babamer Zohra et al., 2012] [20] ; [Allaoui et al., 2014] [16]. Therefore the high activity of the alcoholic extracts of *Cyndon dactylon (L) Pers* roots compared with the dichloromethane and ethyl acetate as shown in the results can be attributed to the presence of phenolic compounds and flavonoids that have inhibitory effect on the positive and negative gram bacteria.

Generally, the four different extracts of this plant are more or less effective towards the tested bacteria and ethanolic/H_2O extracts are more potent compared to ethyl acetate and dichoromethane ether extracts.

CONCLUSION

This study underscored the antimicrobial activity of one chenopodiaceae species namely: *Cyndon dactylon (L) Pers* using four different solvents: Dichloromethane, Ethyl acetate, n-butanol and ethanol/H_2O with increasing polarity against six bacteria strains. The results partially justify the claimed uses of the selected plant in the traditional system of medicine to treat various infectious diseases caused by the microbes. Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plant responsible for the antimicrobial activity.

ACKNOWLEDGMENTS

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MHESR). The authors are thankful to Dr. Ouassila Mokhtari, Mohammed Nadjib Ben Fatah, Hamid Zardouhi and Ahmed Boulaaziz, Arris Hospital, Batna; Dr. A. Khalil, Hakim Saadan Hospital, Biskra ; Mdame Kourim and Mr. Berrah, Mohammed Bouthiaf Hospital, Ouargla 30000, Algeria for their assistance and providing the necessary facilities to carry out this work.

REFERENCES

[1] Chakra borty , P., Urinary tract infection: Text book of microbiology st. ed–new central book agency, Calcutta, India, P ; (1996) 577 -581.

[2] Funfstuck, R., Smith, J. W., Tschape, H. and Stein, G., pathogentic aspects of uncomplicated urinary tract infections, recont advances. clin. Neph, (1997) 47(1) :13-18.
[3] Lettgen, B., Urinary tract infections in childhood old and aspects. klin. pediatr; (1993) 205 (5): 325- 331.
[4] Strffon, R . A., Urinary tract infection problem in diagnosis and management . Med . Clin . North Am, (1974) 58(3) : 545–553.
[5] Egorove , N. S., Antibiotics scientific Approach .Mirpublis hers Moscow. Antimicrob – chemoth (1985).
[6] Kaliyaperumal, Ashokkumar, K. S., Saradha D. M., J. Med. plants Res., (2013) 7 (48) : 3477-3483.
[7] Kirtikar K. R., Basu, B. D., Indian Medicinal Plants, Allahabad, India International book distributer, (1996) Vol 4, 2nd Edn., 1020.
[8] Karthik D., Ravikumar R., Proteome and phytochemical analysis of Cyndon dactylon leaves extract and its biological activity in diabetic rats, Biomed. Rev. Nutr., (2011) 1 (1) : 49-56.
[9] Oudhia, P., Medicinal Weeds in rice fields of chhattisarh (Idia). Int. Rice Res. (1999) 24 (1) : 40-41.
[10] Ahmed, S., Reza, M. S., Jabbar, A., Antimicrobialactivity of Cyndon dactylon, Fitoterapia, (1994) 65 : 463-464.
[11] Kanimozhi, D., Ratha, B. V., In vitro anticancer activity of ethanolic extract of Cyndon dactylon agaist HT-29 cell line. Int. J. Curr. Sci., (2013) 5: 74-81.
[12] Winberg, J., urinary tract Infections in children curr., opin. Inf. Dis., (1990) 3: 55-61.
[13] Colombo, M. L., Bosisio, E., Pharmacological activities of Cheldonium majus L (Papaveraceae). Pharmacol Res., (1996) 33: 127-131.
[14] Iwu, M.W., Ducan, A. R., and Okunji, C. O., New antimicrobials of plant origin. In; Janick J. ed. Perspictives on new Crops and new Uses. Alexandria, VA; ASHS Press, (1999) 457.
[15] Benkeblia, N., Antimicrobial activity of essential oil extracts of various onions (allium cepa) and garlic (Allium stivum). Lebensm-Wiss u-Technol, (2004) 37: 263-267.
[16] Allaoui, A., Cherity. A., Al-Gharabli.S., Gherraf, N., Chebouat, E., Dadamoussa, B., A. Al-Lahhm, A., Research Journal of pharmaceutical Biological and Chemical Sciences, (2014) 5 (5), 85-89.
[17] Ashakkumar, R., and Ramaswamy, M. J., Chem Bio Physci Sec., (2013) 3(2): 1279.
[18] Bindu. J., Anjali. K., and Vibhor K.j., Asian Journal of Biochemical and Pharmaceutical research, (2011) 2(1): 437-442.
[19] Mukundam, B., Shagufa, A., and Swarnamoni, D. A., Asian J. Pharm. Bio res., (2012) 2(3) : 183-188.
[20] Babamer Zohra Y., Sekhri, L., Al-Jaber, Hala I., Al-Qudah, Mahmoud A., and Abu Zarga, Musa H. Journal of Asian Natural Products Research, (2012) 1-7.
[21] Geisman, T. A., Chemistry of Havonoids compounds. Macmillan Co. New York. (1962) 90-101.
[22] Al-Abid, M. R., Zurrzusamme mse turungder Abschla B membrane in Phoenix dactylifera. Wurzburg University. Wurzburg, F. R. of Germany, (1985) 153-140.
[23] Winberg, J., Urinary tract infections in children curr. Opin. Inf. Dis., (1990) 3 : 55-61.
[24] Harbone, J. (1973). Phytochemical methods. Chapman and Hall. London.
[25] Oguyemi AO. In: Sofowora A. ed. Prodeedings of a Conference on African Medicinal plants. Ife-Ife: Univ Ife, (1979) 20-22.
[26] Harbone, J. B., (1984). Phytochemical methods. A guide to modern techniques of p lant analysis. 2nd ed., Chapman and Hall. London. P: 288.
[27] Swarnamoni, D., Mukundam, B., and Shagufa, A., Asian Pharm Clin Res., (2013) 6 (4) : 136-139.