Vol 7 | Issue 1 | 2016 | 51-56.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

ESTIMATION OF POLYPHENOLS AND FLAVONOIDS CONTENTS AND EXPLORATION OF ANTIMICROBIAL NATURE OF *TECOMA STANS* EXTRACTS

R.Sundara Ganapathy[1], S.Mohan[2], S.Kameshwaran[3*], C.Dhanapal[4]

[1]Dean, Faculty of Pharmacy, Karpagam Academy of Higher Education (Deemed to be University), Eachanari Post, Coimbatore, Tamilnadu, India.
[2]Principal, Karpagam College of Pharmacy, Othakkalmandapam, Coimbatore, India
[3]Department of Pharmacology, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS, Ponekkara P. O, Kochi, Kerala, India.
[4]Departmentof Pharmacology, JKK Munirajah College of Pharmacy, Kumarapalaym, Namakkal, Tamilnadu, India.

ABSTRACT

This study aimed to evaluate the antibacterial activity of *Tecoma stans* extracts from two Tamilanadu regions and to determine their total polyphenol's and flavonoid's contents. The disk diffusion method was used to evaluate antimicrobial activity. Folin Ciocalteu and aluminum trichloride methods were used to determine the Total polyphenol's and flavonoid's contents respectively. The ethyl acetate extracts induced inhibition zone diameters of 21.52 mm and 19.8 mm for samples collected from Namakkal (NK) and of 19.8 and 19 mm for those from Madurai (MD) respectively in *Mycobacterium aurum* and *Mycobacterium smegmatis*. Inhibition zone diameters of *Pseudomonas aeruginosa* were only 11.98 and 9.96 mm respectively for NK and MD ethyl acetate extracts. The polyphenols and flavonoids contents were respectively 608.74µg/ml and 104.84 µg/ml in the MD ethyl acetate extract and only 474 and 5.74 µg/ml in the dichloromethan extract. The antibacterial activity of tested extracts depends on the extract's nature, the bacterial strain and the plant's geographical provenance. The ethyl acetate's extract of *T.stans* from NK was the most active. *Mycobacterium aurum* and *M. smegmatis* were the most sensitive to *Tecoma stans* extracts and *P. aeruginosa* was the most resistant. The polyphenol's and flavonoid's contents were different depending on the extract's nature and the plant's provenance. Valorization of *Tecoma stans* and evaluation of its biological and phytochemical activities will enable us to screen and test new natural antibacterial molecules in general and antimycobacterial agents in particular.

Keywords: Antibacterial activity, *Tecoma stans*, *Mycobacterium*, Total polyphenols, Flavonoids.

INTRODUCTION

For centuries, plants have been used to treat several diseases [1]; hence most eminent doctors were herbalists in the past. Currently, medicinal and aromatic plants have considerable advantages due to the progressive discovery of their applications in health care as well as their uses in other domains of economic interests.

Because of these numerous uses they know a stronger demand on the world market. The antimicrobial properties of medicinal plants were recognized for a long time, but were confirmed scientifically only recently. These plants have an enormous therapeutic potential to treat many infectious diseases [2]. Indeed, they contains numerous substances able to protect against microorganisms, insects and weeds [3]. So, the plant's production of secondary metabolites, having an antibacterial activity, took an important place in research studies [4, 5]. Several researchers studied the biological activities of aromatic and medicinal plants from different world's regions. Some extracts were effective to control the growth of a big variety of microorganisms.

Tecoma stans (Common name: Yellow bell) is also known as yellow trumpet bush and belongs to the family Bignoniaceae. It is an erect ornamental plant and is a branched, slightly hairy or nearly smooth shrub 2 to 4 m in height. The leaves are opposite, odd-pinnate and up to 20 cm in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong-lanceolate, 6 to 13 cm long, pointed at both ends and toothed on the margins.

Corresponding Author: **S.Kameshwaran** **Email:-** kameshwar21846@aims.amrita.edu

The trumpet-shaped flowers are yellow, faintly scented and occur in short, dense, terminal clusters. The calyx is green, 5 to 7 mm long and 5-toothed. Flowering can begin as early as April and continue into the fall (autumn)[6].

The leaves of T. stans contain the alkaloids tecomine and tecostamine, potent hypoglycaemic agents when given intravenously. Anthranilic acid is responsible for its antidiabetic activity and the roots exhibit a powerful diuretic and vermifuge activity[7]. Tecoma is not toxic and is used in Latin America as a remedy for diabetes and also for feeding cattleand goats in Mexico [8]. In this context, this study was conducted in a perspective to evaluate and valorize the phytochemical activity of *T.stans* even if this plant has no medical or medicinal previously known uses; however, it is used in tanning, burning and utensil washing. Hence, the aim of our study was to evaluate the antibacterial activity of *Tecoma stans* extracts against *Mycobacterium smegmatis*, *M. aurum*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Bacillus* spp., *Enterococcus faecalis*, and to determine their total contents of polyphenols and flavonoids. In addition, it represents a contribution for providing prevention or alternative treatment for chronic and/or severe infectious diseases, and for solving the problem of bacterial resistance against existing antibacterial agents. Moreover, the evaluation of antimycobacterial activity of this plant has never been investigated.

MATERIALS AND METHODS

Plant material

Tecoma stans was collected, in February 2015, from two different stations in Tamilnadu (Namakkal and Madurai). The samples taken to laboratory were air-dried, then leaves were separated from stems and grind to a suitable particle size for optimal dissolution.

Bacterial strains tested

Several gram-positive and gram-negative bacteria were used to test the antibacterial activity of *Tecoma stans* extracts: *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Salmonella* sp., *Enterococcus faecalis*, *Mycobacterium smegmatis* MC²155 and *Mycobacterium aurum* A+. The tested bacteria are pathogenic and are known for their invasiveness and toxicity to humans. They are frequently encountered in many infections like tuberculosis (*Mycobacterium*), food borne illness (*Salmonella*), urinary infections (*Enterococcus*); skin, respiratory, endovascular infections (*Staphylococcus aureus*) and also Nosocomial infections (*Staphylococcus*, *Pseudomonas*). Some ones are also responsible of food alteration (*Bacillus*).

Preparation of extracts

Extraction was performed by Soxhlet or by sonication of the *Tecoma stans* leaves powder. Hence, successive application of four analytical solvents (hexane, ethyl acetate, dichloromethane and methanol) to 45 g of powder put in the cartridge of the Soxhlet, yielded various extracts using the protocol described by Farrapo *et al.* (2011) [9]. For the second method, 45 g of powder was immersed in 200 ml of methanol and then subjected to ultrasonication (30 °C, 35 KHz) [5]. At the end of each extraction (Soxhlet, ultrasonication), the solvents were evaporated using a rotary evaporator (90 rpm, 40 °C). Each collected extract was named depending on the solvent used during its extraction.

Evaluation of the extract's antibacterial activity

The study of antibacterial activity was performed by the agar diffusion method to select the most active extract. Sterilized Whatman paper discs of 6 mm diameter were placed in Petri dishes previously seeded with the tested strain, then impregnated with 10 µl of one of the previously prepared extract. The inhibition zone diameters were measured after incubation at 37 °C, 72 h for Mycobacterium species and 24 h for the other strains. Solvent impregnated discs were used as controls. Which were performed in the same experimental conditions of the tests. Three repetitions were performed for each test [10,11].

Determination of the minimum inhibitory concentration (MIC)

The agar dilution technique was used to determine the Minimum Inhibitory Concentration (MIC) of the tested extract [11]. This technique consists in seeding, by a standardized inoculum, a serial dilution of extracts contained in a liquid suitable medium. After incubation, growth observation enabled us to determine the MIC, which is the lowest concentration of extract able to inhibit bacterial growth. Tubes containing 2 ml of sterile LB-agar media were prepared and maintained in a water bath at a temperature of 48 °C to 50 °C. 2 ml of the extract's dilution prepared in LB agar medium was added to each tube, and after homogenization the mixture was poured into sterile Petri dishes. 5 µl of each inoculum (10^8UFC/ml) were seeded as a spot on the surface of the agar well dry. The dishes were then dried at room temperature until the medium completely absorbs inoculums then incubated at 37 °C. The range of final extract dilution used was 160 to 2.5 mg/ml.

Determination of flavonoids and polyphenols contents

The determination of total polyphenol's contents in extracts was made according to the Folin Ciocalteu method [12] by measuring the absorbance at 765 nm against a control without extract. The total polyphenol's content in the four extracts (at final concentration 1 mg/ml) was calculated from a linear calibration curve (y = ax+b), established with gallic acid (0-500 µg/ml) as a standard reference in the same conditions as the sample. While the aluminum trichloride method was used to determine the total flavonoids content in the tested extracts [13]. Hence, 1 ml of each extract sample at (1 mg/ml) was added to 1 ml of $AlCl_3$solution (2% in methanol) then, the mixture was vigorously stirred and incubated 10 min. Finally the absorbance was read at 430 nm. A calibration curve (y = ax+b) established by quercetin (0-40 µg/ml) was performed under the same operating conditions as for the samples and was used for flavonoids quantification. [14]

Statistical analysis

The results were expressed as mean of the three repetitions and standard deviations were calculated. Statis-

tical comparisons were made using the Student's test and $p<0.05$ was considered as significant [15].

RESULTS

Antibacterial activity of the extracts

Results of the antibacterial activity of *T.stans*'s tested extracts, against seven bacterial strains implicated in several pathologies and/or implicated in food alteration process, resulting in the appearance of inhibition zones around the disks are shown in Tables 1 and 2.

The ethyl acetate extracts induced inhibition zone diameters ranging from 16.75 to 21.5 mm and from 9.1 to 19.8 mm for *T.stans* harvested respectively from NK and from MD. While the soxhlet methanol extract induced inhibition zone diameters from 10.82 to 16.9 mm and 7.1 to 16.01 mm for NK's and MD's harvested plants respectively. Dichloromethan extracts induced, for all the tested strains, the lowest inhibition zone diameters (6 to 10.4 mm) which were very close to controls. Moreover, the ethyl acetate (EA) extracts induced inhibition zone diameters of 21.52 mm and 19.8 mm for samples collected from NK and of 19.8 and 19 mm for those from MD respectively in *Mycobacterium aurum* and *M. smegmatis*. While, inhibition zone diameters of *Pseudomonas aeruginosa* were only 11.98 and 9.96 mm respectively for NK and MD ethyl acetate extracts. The inhibition zone diameters of *S. aureus* and *E. faecalis* by the EA extract from NK's plants were respectively 14.89 and 16.85 mm, while those of MD's EA extract didn't exceed 11 mm. But they ranged between 11 and 12 mm with the soxhlet methanol extract for the same strains.

Flavonoids and polyphenols content determination

Results of Flavonoïds and polyphenols contents in the four extracts selected according to their antibacterial activity are shown in table 4. The highest polyphenols and flavonoids concentrations were found in the ethyl acetate extract from MD's harvested plants (about 608.74±6 µg/ml and 104.84±0.5 µg/ml respectively), followed by those of the methanolic extract obtained by soxhlet from NK (489.7±3.3 and 33.75±0.52 µg/ml respectively), while the lowest concentrations were observed for the dichloromethane extract from NK (474.3±3.58 and 5.74±0.51 µg/ml respectively).

Table 1. Antibacterial activity of the extracts of *Tecoma stans* collected from NK (Inhibition zone diameters in mm)

Strains	T1	T2	T3	A	B	C	D
Salmonella	6.89±0.04	8.99±0.12	7.88±0.21	16.75, 1±0.21**	10.86±0.21	10.82, 2±0.12	8.4±0.21
Pseudomonas aeroginosa	7.68±0.21	5.84±0.94	6.85±0.94	11.98±0.24*	7.86±0.25	7.96±0.14	6.85±0.51
Staphylococcus sp.	6.98±0.21	7.83±0.12	6.98±0.2	14.89±0.21**	9.58±0.21	10.99±0.14	8.5±0.94
Bacillus sp.	6.89±0.14	6.91±0.41	6.86±0.11	15.97±0.10**	9.56±0.21	11.89±0.21	9.85±0.54
Enterococcus faecalis	5.96±0.12	5.89±0.14	5.84±0.84	16.85±0.2**	10.85±0.21	11.58±0.10	9.01±0.54
Mycobacterium aurum	9.01±0.15	10.02±0.14	8.02±084	21.5±0.24**	14.85±0.24*	14.9±0.8*	9.9±0.12
Mycobacterium smegmatis	11.4±0.10	11.2±0.11	11.4±0.20	19.8±0.12**	18.4±0.21*	16.9±0.54*	9.9±0.20

T1: Ethyl Acetate Control, T2: Methanol Control, T3: Dichloromethan Control, A: Ethyl acetate extract, B: Methanol extract obtained by sonication, C: Methanol extract obtained by Soxhlet, D: dichloromethane extract.

Table 2. Antibacterial activity of the extracts of *Tecoma stans* collected from MD (Inhibition zone diameters in mm)

Strains	T1	T2	T4	A	B	C	D
Salmonella	6.9±0.10	8.94±0.17	8.01±0.78	9.1±0.29	9.01±0.61	11.94±0.40*	8.01±0.47
Pseudomonas aeroginosa	8.1±0.54	6.10±0.21	7.01±0.64	9.96±0.12*	5.87±0.10	11.98±0.17**	6.89±0.12
Staphylococcus	7.01±0.24	8.1±0.78	7.1±0.64	11.01±0.15**	9.94±0.15	11.01±0.14*	7.1±0.14
Bacillus sp	6.98±0.48	7.01±0.24	7.02±0.84	9.99±0.84	7.01±0.14	7.1±0.04	7.01±0.14
Enterococcus faecalis	6.1±0.14	6.02±0.74	6.01±0.84	11.01±0.24**	8.3±0.15	11.01±0.31*	6.21±0.15
M. aurum	8.99±0.11	9.94±0.14	8.14±0.11	19.8±0.25**	10.01±0.24	14.89±0.28*	8.01±0.12
M. smegmatis	10.4±0.17	10.01±0.87	10.24±0.98	19.0±0.84**	10.07±0.21	16.01±0.25*	10.04±0.84

T1: Ethyl Acetate Control, T2: Methanol Control, T3: Dichloromethan Control, A: Ethyl acetate extract, B: Methanol extract obtained by sonication, C: Methanol extract obtained by Soxhlet, D: dichloromethane extract.

Results of the most active extract's Minimum Inhibitory Concentration, determined by the agar dilution method, showed that the highest MIC value (80 mg/ml) was obtained for *Pseudomonas aeruginosa* by the ethyl acetate extract of NK, followed by *Salmonella* sp. (40 mg/ml) then the other strains (20 mg/ml), while the lowest value was obtained for *Mycobacterium smegmatis* (10 mg/ml) (table 3).

Table 3. MIC of the Ethyl Acetate extract of *Tecoma stans* from NK

Strains	2.5	5	10	20	40	80	160
Enterococcus faecalis	+	+	+	-	-	-	-
Salmonella sp.	+	+	+	+	-	-	-
Staphylococcus aureus	+	+	+	-	-	-	-
Bacillus	+	+	+	-	-	-	-
Pseudomonas aeroginosa	+	+	+	+	+	-	-
Mycobacterium aurum	+	+	+	-	-	-	-
Mycobacterium smegmatis	+	+	-	-	-	-	-

The concentrations are expressed in mg/ml,+Presence of bacterial growth,-Inhibition of bacterial growth.

Table 4. Total content of phenols and flavonoids in different extracts of *T.stans* (in µg/ml)

Extracts	Total polyphenols concentration (µg/ml)	Total flavonoïds concentration (µg/ml)
1B	479.98±2.60	16±0.58
2B	608.74±6.84	104.84±0.54
3B	489.78±3.34	33.75±0.52
4B	474±3.58	5.74±0.51

1B: Ethyl acetate extract of *T.stans* from NK, 2B: Ethyl acetate extract of *T.stans* from MD, 3B: Methanol extract obtained by Soxhlet (from NK), 4B: Dichloromethane extract (from NK).

DISCUSSION

The ethyl acetate (EA) extracts of *T.stans* induced the greatest inhibition zones diameters, followed by methanol extracts obtained by Soxhlet method. While the inhibition zone diameters obtained with the Dichloromethane extracts were very low for *T.stans* collected from NK and very close to the controls for those from MD. Moreover, we noted that the antibacterial activity of *T.stans*'s extracts from NK was higher than that of MD's extracts, tested in the same operating conditions.

The antibacterial activity of different *T.stans*'s extracts found against several gram positive and gram negative strains confirm that the studied plant have an important activity and can be used as an inexhaustible source of new natural antibacterial agents, which is concordant with our previous work on its aqueous extracts [14].

Staphylococcus aureus and *Enterococcus faecalis* were highly sensitive to the EA extracts and slightly sensitive to the methanolic extract of *Tecoma stans* from both stations. *Mycobacterium aurum* and *M. smegmatis* were the most sensitive tested strains to all the studied extracts (most inhibited), with a strong activity observed for the ethyl acetate extract ($p<0.05$). A previous study conducted by Boudkhili *et al.* (2012) [15] also showed that *Tecoma stans* has an important antibacterial effect against *Staphylococcus* sp. and *Salmonella* sp. However, the Coriaria's antimycobacterial activity and its activity against the remaining tested bacterial strains have never been tested nor demonstrated. Furthermore, another preliminary study, measuring the antimycobacterial effect of species belonging to different families such as *Asparagus stipularis, Cyperuslongus, Scirpoides holoschoenus, Charybdis maritima, Dipsacus fullonum, Teucrium fruticans*, indicated that these plant's extracts at a concentration of 160 mg/ml were inactive against *Mycobacterium aurum* and *M. smegmatis* [10]. While the same dose of *Tecoma stans* ethyl acetate extract of NK was found in our study to be bactericidal against these strains and showed the lowest MIC value for *M. smegmatis*.

These results show a promising antimycobacterial effect of the tested extract which should be more studied and valorized to determine the bioactive phytomolecules involved in this activity. The inhibition zone diameters values obtained for *Staphylococcus aureus* and *Enterococcus faecalis* showed their important sensibility to *Tecoma stans*'s extracts, especially the ethyl acetate one, which was confirmed by the low MIC values (20 mg/ml).

Based on the comparison between the different extracts action's results, we can note that the ethyl acetate extract and methanol extract obtained by Soxhlet method were the most active, while the dichloromethane extracts have the lowest inhibitory activity in plants collected from both stations. Indeed, the EA extracts of the two samples have a wide action spectrum both on gram-positive and gram-negative bacteria. This result is concordant with those of a previous study investigated by Bolou *et al.* (2011) [16] on the EA extracts of *Terminalia glaucescens*.

Antimicrobial activity differences observed against tested germs between these extracts could be explained by the nature and/or the concentration of molecules contained in each one of them. This confirms the existence of differences in solubilization capacity of solvents used in the extraction of active phytomolecules that have been reported in a previous study [16].

The significant difference in antibacterial activity, ($p<0.05$), between extracts of the tested plants collected from two stations can be explained by the influence of environmental conditions on the plant's metabolism according to Aganga and Mosase's findings [17].

Comparison of MIC results of Ethyl acetate *T.stans*'s extract against all tested strains showed that *Pseudomonas aeruginosa* was the most resistant one, while *Mycobacterium smegmatis* was the most sensitive.

The polyphenol's and flavonoid's contents determination showed that their concentrations in the ethyl acetate's extract from NK were lower than those from MD. This content's difference between the two samples can be explained by the environmental condition's influence on

the plant metabolism [17]. The difference in flavonoid's and polyphenol's contents obtained between the three tested extracts suggest that ethyl acetate and methanol can solubilize the maximum of chemical compounds in comparison with dichloromethane which showed the lowest concentrations of the two compounds. Indeed, previous studies have shown that the extraction of bioactive components depends not only on the extraction's time and temperature, but mainly on the nature of extraction solvent used [18,19]. In addition, the great antibacterial activity of ethyl acetate and methanol extracts could be related to their important content of polyphenols and flavonoids which could correspond to the main active compounds responsible of this activity. Furthermore, we found that the ethyl acetate extracts were more active than the methanolic ones, although both were rich in polyphenols and flavonoids. This suggests the presence of a synergic effect between these phenolic compounds and other plant components, according to previous findings and studies showing that the plants antibacterial activity depends not only on the presence of phenolic compounds, but also the presence of a variety of secondary metabolites [20, 21]. Moreover, this can be confirmed by the low activity of MD plant's extracts in spite of their high polyphenol's and flavonoïd's contents which suppose that they're not the only active compounds. Indeed, *T.stans* leaves are known to contain tannins which can also contribute to their antimicrobial activity.

CONCLUSION

In this study, we evaluated the antibacterial activity of *Tecoma stans* extracts from two different stations in Tamilnadu, against seven gram positive and negative bacterial strains implicated in several pathologies and/or implicated in food alteration process on one hand. On the other hand, we determined the contents of polyphenols and flavonoids of these *Tecoma stans* extracts The tested extracts presented variable antibacterial activity against the seven pathogenic tested strains (*Salmonella* sp., *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp., *Enterococcus faecalis, Mycobacterium aurum* and *Mycobacterium smegmatis*). The ethyl acetate's extract of *T.stans*, collected from NK, was the most active and was selected for determining its Minimum Inhibitory concentration. The polyphenols and flavonoids concentrations were different depending on the extract's nature and the plant's provenance and were important in the ethyl acetate and the methanolic extracts. The significant antibacterial activity of the studied extracts can be explained by their active compounds. Finally, highlighted performances of *Tecoma stans* extracts encourage the valorization of this plant, by evaluating other biological and phytochemical activities and by revealing his chemical composition to determine the active biomolecules. Moreover, it opens a perspective for screening and testing new antimycobacterial agents.

ACKNOWLEDGEMENT: None.

CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

1. Djeridane A, Yousfi M, Nademi B, Boutassouna D, Stocker P, Vidal N, Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*, 97, 2006, 654-660.
2. Janovska D, Kubikova K, KMDosba L, Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. *Czech J Food Sci*, 21, 2003, 107-110.
3. Thuille N, Fille M, Nagl M, Bactericidal activity of herbal extracts. *Int J Hyg Environ Health*, 206, 2003, 217-221.
4. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev*, 10, 1999, 564-582.
5. Vuotto LM, Basile A, Moscatiello V, Sole DP, Castaldo-Cobianchi R, Laghi E, Antimicrobial and antioxidant activities of *Feijoa sellowiana* fruit. *Int J Antimicrob Agents*, 13, 2000, 1997-2001.
6. Sugavanam, K, Velayutham s, Ganesan A, CNS depressent activity of different extracts of *Tecoma stans* flowers. *Asia. J. Trad. Med*, 7, 2012, 39-43
7. Khare, C.P, Indian Medicinal Plants and Illustrated Dictionary. Springer Science Publishers, New Delhi, India, 2007.
8. Susano Hernandez, R, Especies arboreas forestalessusceptibles de aprovecharse como forraje. *Ciencia. Forestal*, 6, 2007, 31-39
9. Farrapo MN, Gleidy Silva AA, Karine N, Costa, Magali Silva G, José Cogo C, Inhibition of *Bothropsjararacussu* venom activities by *Plathymenia reticulate* Benth extracts. *J Venom Res*, 2, 2011, 52-58.
10. Sqalli H, El Ouarti A, Ennabili A, Ibnsouda S, Farah A, Haggoud A, Evaluation de l'effet antimycobactérien de plantes du centre-nord du Maroc. Bull Soc Pharm Bordeaux, 146, 2007, 271-288.
11. NCCLS, National committee for clinical laboratory standards. Performance standards for antimicrobial disk susceptibility tests, Approved standard M02-A11. 11thed. Wayne, Clinical and Laboratory Standards Institute, 2012, 67.
12. Singleton LV, Orthofer R, Lamuela-Raventos MR, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods Enzymol*, 299, 1999, 152-178.
13. Warburton E, Norris LP, Goenaga-Infante H, Comparison of the capabilities of accelerated solvent extraction and sonication as extraction techniques for the quantification of kavalactones in *Piper methysticum* (Kava) roots by high performance liquid chromatography with ultra violet detection. *Phytochem Anal*, 18, 2007, 98-102.
14. Hezbri K, Hafsé M, Farah A, Sbissi I, Gtari M, Fikri Benbrahim K, Antibacterial activity of lyophilized aqueous extract of *Tecoma stans* from Northern Morocco. J Adv Biotechnol, 3(3), 2014, 280-285.
15. Boudkhili M, Greche H, Bouhdid S, Zerargui F, Aarab L, *In vitro* antioxidant and antimicrobial properties of some Moroccan's Medicinal plants. *Int J Pharm Tech Res*, 4, 2012, 637-642.

16. Bolou EGK, Attiqua B, N'Guessan AC, Coulibaly A, N'Guessan JD, Djaman AJ, Évaluation *in vitro* de l'activité antibactérienne des extraits de *Terminalia glaucescens planch*. sur *Salmonella typhi* et *Salmonella typhimurium*. *Bull Soc R Sci Liege*, 80, 2011, 772-790.
17. Aganga AA, Mosase KW, Tannins content nutritive value and dry matter digestibility of *Lonchocarous capussa, Ziziphus mucropata, Sclerocarya birrea, Kirkia acuminata and Rhus lancea* seeds. *Anim Feed Sci Technol*, 691, 2001, 107-113.
18. Turkmen N, Sari F, Velioglu SY, Effects of extraction solvents on concentration antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chem*, 99, 2006, 835-841.
19. Bazinet L, LaNKe D, Tremblay A, Production of green tea EGC-and EGCG-enriched fractions by a two-step extraction procedure. *Sep Purif Technol*, 56, 2007, 53-56.
20. Cetkovic GS, Canadovic-Brunet JM, Djilas MS, Tumbas TV, Markov LS, Cvetkovic DD, Antioxidant potential, lipid peroxidation inhibition and antimicrobial activities of *Satureja montana L. subsp. Kitaibelii* extracts. *Int J Mol Sci*, 8, 2007, 1013-1027.
21. Stefanakis MK, Touloupakis E, Anastasopoulos E, Ghanotakis D, Katerinopoulos EH, Makridis P, Antibacterial activity of essential oils from plants of the genus *Origanum*. *Food Control*, 34, 2013, 539-546.