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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *TAMUS COMMUNIS L.* ROOT EXTRACTS FROM ALGERIA

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ABSTRACT

The present study was conducted to evaluate the in vitro antioxidant and antibacterial properties of *Tamus communis L.* root extracts (TCRE), which is a plant used in traditional medicine in Algeria. The antioxidant potential of pattern was evaluated using two complementary techniques, inhibition of free radical DPPH and the test of β-Carotene / linoleic acid. The antioxidant test indicates that non-polar fractions of TCRE (chloroform and ethyl acetate fractions) were more active than the polar fractions. Among these fractions, the chloroform extract appear in the DPPH test an IC50 of (0.0189 mg/ml) comparable to that of BHT (0.0186 µg/ml). This fraction was able to inhibiting the oxidation of β-Carotene with a percentage of inhibition (89.84 %). In antibacterial test, non-polar fractions showed antibacterial activity very important compared with the polar fractions. These fractions have inhibited the growth of four from nine bacterial strains, causing zones of inhibition from 08 to 14 mm of diameter.

INTRODUCTION

Long time before the humanity discovered the existence of microbes, the idea that certain plants had healing potential been well accepted (Rıos and Recio, 2005), and several different lines of evidence indicate that medicinal plants represent the oldest and largest form of medication, they were regularly used by people in prehistoric times (Rıos and Recio, 2005). These beneficial health effects have been attributed in part to the presence of bioactive compounds in dietary plants, which may exert their effects as a result of their antioxidant properties e.g. vitamin C, vitamin E, carotenoids, polyphenolic compounds and flavonoids Barros *et al*., 2007). Recently, interest has increased considerably in finding naturally bioactive compounds for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Ito *et al*., 1983), (Kumaran and Karunakaran, 2007). Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Halliwell, 1997), (Halliwell and Halliwell, 1999). Among the various natural antioxidants, Polyphenolic compounds play an important protective effect mainly due to their large array of biological actions such as free radical-scavenging, metal chelation, peroxides decomposition and enzyme modulation abilities, as well as their effects on cell signaling pathways and on gene expression (Soobrattee *et al*., 2005), (Agli *et al*., 2005), (Rodrigo *et al*., 2011). Their presence in the diet appears to be associated with lower occurrence and lower mortality rates of several human diseases (Andersan *et al*., 2001), (Ksouri *et al*., 2007). Phenolic compounds also exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, anti-thrombotic, cardioprotective and vasodilatory effects (Balasundram *et al*., 2006). Also, phenolic compounds of plant materials including vitamins, pigments and flavonoids, possess potential antibacterial (Baydar *et al*., 2006), antifungal (Bruno, and Sparapano, 1999), antiviral (Chavez *et al*., 2006) and antimutagenic properties as well as blood glucose decreasing activity (Thompson *et al*., 1984). *Tamus communis L*, called El-Karma Saouda (fam. Dioscoreaceae) is a climbing plant with large tubers which causes irritation when rubbed on the skin (Schmidt, and Schmidt, 1983). It displays a broader distribution in north of Algeria (Boumerfeg *et al*., 2009) and in Europe (Kovacs *et al*., 2007). Both the rhizomes and the berries have been reported to be used in folk medicine as effective treatment of rheumatism, arthrosis, lumbago and dermatosis (Duke, 2002). Moreover, different parts of the plant have been applied in traditional medicine for the treatment of polyps and tumours (Hartwell, 1969).

Previous phytochemical investigations revealed the presence of spirostane and furostane glycosides, sterols, histamine and hydroxy/alkoxy- substituted phenanthrenes and dihydrophenanthrenes (Kovacs *et al*., 2007). The aim of the present study was to investigate the potential of *Tamus*

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communis L root extracts to act as an antioxidant, free radical scavenging material and to examine their antibacterial effects.

MATERIALS AND METHODS

Collection of plant material

The roots of *Tamus communis L*, were coolected from BouGaa Wilaya of Setif, northeast of Algeria, Authenticated by Dr Daniel Jeanmonod, conservator, Geneva University, Switzerland. A voucher specimen was deposited at the Laboratory of Botany, Department of Biology, Faculty of Sciences, University Ferhat Abbas Setif, Algeria.

Chemicals

Methanol, hexane, chloroform, ethyl acetate, Tween 40 and HCl (Merck); 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), β–carotene, linoleic acid, Mueller Hinton Agar, Potassium ferricyanide [K3Fe (CN)6], Ferric chloride (FeCl3), Aluminium chloride (AlCl3), Diméthyle sulphoxide (DMSO), phosphoric acid (H3PO4) (85%). (Sigma, Aldrich or Fluka). Gallic Acid, quercetin, rutin, BHT (butylated hydroxytoluene), Antibiotics: Polymyxin (PB), Neomycin (Neo), Nibiol (Nib), Doxycylin (Dot), Cloramphenicol (Cmp), Gentamicin (Gen), Erythromycin (E), Amoxicillin + Clavulanic acid (AMC), Penicillin (Pen), Cefazolin (CZ), Amoxicillin (AMX), Lincomycine (Lin), Sulfamid Forts (Suf), Cefotaxim (CTX) (Bio–Rad).

Preparation of the Tamus communis L. root extracts (TCRE)

The extracts were prepared according to the method of Markham (Markham, 1982), which is based on the solubility degree of flavonoïds in organic solvents. For obtained Crud extracts (CE), the roots dried plant material (broyat) was submit to an extraction with a hydroalcoholic mixture methanol/water. The aqueous solution was extracted with hexane several times to eliminate lipids. The water fraction was partitioned against chloroform labelled CHE. The remaining aqueous phase was extracted exhaustively with ethyl acetate until the final extract was colourless (fraction labelled EAE), the remaining aqueous extract was labelled AE. All the solvents were removed by evaporation under reduced pressure and the extracts were stored at −20 °C until use.

Determination of total polyphenols content

Total polyphenols were measured using Prussian blue assay method descriebed by Price and Butler (Price and Butler, 1977) and modified by Graham (Graham, 1992). Phenolic contents were expressed as gallic acid equivalents. Briefly, 0.1 ml of TCRE dissolved in methanol and 3 ml distilled water were added and mixed up. One ml of K3Fe (CN)6 (0.016 M) was added to each sample followed by addition of 1 ml of $FeCl_3$ (0.02 M dissolved in 0.1 M HCl) and immediately mixed up using a vortex. After the addition of the reagents to the sample, 5 ml stabilizer (1/5 of 1 % gum Arabic, 1/5 of 85 % H_3PO_4 and 3/5 distilled water) were added to the sample and mixed. The absorbance was measured at 700 nm using a UV/VIS-8500 Techom spectrophotometer. The amount of total polyphenols in different extracts was determined from a standard curve of gallic acid ranging from 0.00 to 200 µg / ml.

Determination of flavonoids content

Flavonoids were measured by AlCl3 method described previously by Bahorun (Bahorun *et al*., 1996) and expressed as quercetin equivalents. One ml of the plant extracts samples was dissolved in methanol, 1 ml of AlCl3 (2 % in methanol) was added. After incubation for 10 min, the absorbance was measured at 430 nm.

Free radical scavenging capacity

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Burits and Bucar, 2000). 50 µL of various concentrations of the extracts in methanol was added to 5 mL of a 0.004% methanol solution of DPPH. All determinations were performed in triplicate. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition percent (I%) of the free radical DPPH was calculated according to the following formula:

I% = (A blank - A sample /A blank) x 100

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and butylated hydroxytoluene (BHT) was used as positive controls.

β-Carotene-linoleic acid assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius and Venskutonis, 1998). A stock solution of b-carotene/linoleic acid was prepared as follows: first, 0.5 mg of b-carotene was dissolved in 1ml of chloroform (HPLC grade), then 25 µl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator. Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 µl portions of the extracts (2 g/l in methanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 µl of ethanol. After the incubation period, the absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank (H_2O and CH_3OH).

Antibacterial assay

TCRE were individually tested against a panel of bacteria; Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 2592 3, Bacillus sp, Klebsiella pneumoniae, Salmonella typhi, Enterobacter agglomerans, Serratia marcescens, Citrobacter freundii. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA). For the determination of antibacterial activities, agar well-diffusion method was employed. From bacterial cultures of six hours in nutrient broth with shaking at 37°C, dilutions were made in sterile saline (10-1, 10-2), 5 ml of the dilution (10-2) of each strain are paid on the solidified medium in Petri dishes (MHA) and excess is removed after 2-3 min (method of flooding). The water-soluble and water-insoluble fractions of TCRE were weighed (100 mg each) and dissolved in distilled water (1.0 ml) and dimethylsulphoxide (DMSO) (1.0 ml) respectively, making up the final testing concentration of each extract as 100 mg/ml. The wells (8 mm in diameter) were cut from the agar and 0.06 ml of extract solution was delivered into them. After incubation for 24 h at 37 °C, all plates were examined for any zones of growth inhibition, and the diameter of these zones were measured in millimeters. All the tests were performed in duplicate. All determinations were conducted in triplicate or more and all results were calculated as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phenolic compounds and flavonoids. The quantitative determination of total polyphenols and flavonoids in TCRE are shown in Table 1. There was a wide range of phenol concentrations in TCRE. The value varied from 4.67 ± 0.62 to 78.22 ± 2.25 mg equivalent gallic acid/g lyophilizate and from 1.29 ± 1.01 to 10.27 ± 4.89 mg equivalent quercetin/g lyophilizate for polyphenols and flavonoids, respectively. The highest phenol content was found in CHE followed by EAE, while the total polyphenol levels were particularly low in AE. However, the highest flavonoid content was found in EAE followed by CHE then CE and finally AE. polyphenols and flavonoids content, may differ from one species to another and from one plant organ to another, influenced by several factors such as: geographic location of collection, stage of growth and culture conditions of the plant. Dixon and Paiva, (Dixon and Paiva, 1995), Naczk and Shahidi, (Naczk and Shahidi, 2004), found that the synthesis and accumulation of polyphenols in plants is generally stimulated in response to biotic / abiotic stress as salinity which stimulates the production of phenolic compounds (Ksouri and Megdiche, 2007). Brenda, (Brenda, 2002), announced that plant organs most exposed to UV radiation are richer in flavonoids, such as the epidermal cell layers of leaves, pollen and apical meristem. There is also new evidence which announces that flavonoids are involved in resistance to aluminum toxicity in maize (Kidd *et al*., 2001). The plant roots of maize which are exposed to aluminum have higher amounts of phenolic compounds and 15 times higher in flavonoids when these roots are pretreated by silicone. Scavenging activity on DPPH radical. Because DPPH can be kept indefinitely with little decomposition and because it neither dimerizes nor reacts with oxygen (Blois, 1958), it proved to be quite useful in a variety of investigations, such as determination of antioxidant properties of amines, phenols or natural compounds (vitamins, plant extracts, medicinal drugs) (Blois, 1958). The free radical DPPH_ possesses a characteristic absorption at 517 nm (purple in color), which decreases significantly on exposure to radical-scavengers (by providing hydrogen atoms or by electron donation). A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract. Free radical- cavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the Radical-scavenging activity of specific compounds or extracts (Barros *et al*., 2007).

Results showed that scavenging activity of both TCRE on DPPH was increased as the concentration of extract increased until a plateau where activity stabilizes (Fig 1). The scavenging activity of non polar fractions was significantly higher than polar fractions. IC50 value was determined from the plotted graph of scavenging activity against the concentration of TCRE, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The lowest IC50 indicates the strongest ability of the extracts to act as DPPH scavengers. From values of IC50 (Fig 2) the radical-scavenging effects were excellent for pure compounds: Gallic acid (0.0024 mg/ml), Catechin (0.0044 mg/ml), Cafeic acid (0.0051 mg/ml), Quercetin (0.0054 mg/ml) and Rutin (0.0086 mg/ml), even higher than the scavenging effect of BHT (0.0186 mg/ml). The radical scavenging activity values were very good for the CHE with an IC50 (0.0189 mg/ml) equal to that of BHT, good for EAE (0.0343 mg/ml) and CE (0.1855 mg/ml) and moderate for AE (1.6407 mg/ml) (Fig 3). The scavenger can be explained by the presence of polyphenols and flavonoids

Table 1. total Polyphenol and Flavonoid Contents of Tamus Communis Root extracts (TCRE)

Extracts	Total Polyphenols Mg equivalent gallic acid g-1 lyophilisat	Total Flavonoids Mg equivalent quercetin g-1 lyophilisat
CE	24.756 ± 6.663	02.440 ± 0.440
CHE	78.222 ± 2.251	07.934 ± 0.993
EAE	61.432 ± 9.466	10.265 ± 4.891
AE	04.669 ± 0.616	01.287 ± 1.009

CHE: chloroform extract, EAE: ethyl acetate extract, CE: crud extract and AE: aqueous: extract. Each value represents the mean ± SD (n = 3)

in the extract. Marsden Blois (1958) has recorded nearly 53 years ago that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability (Blois, 1958).

β-Carotene–linoleate bleaching. The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated b-carotene models. The presence of different antioxidants can hinder the extent of b-carotene-

bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha *et al*., 2001).

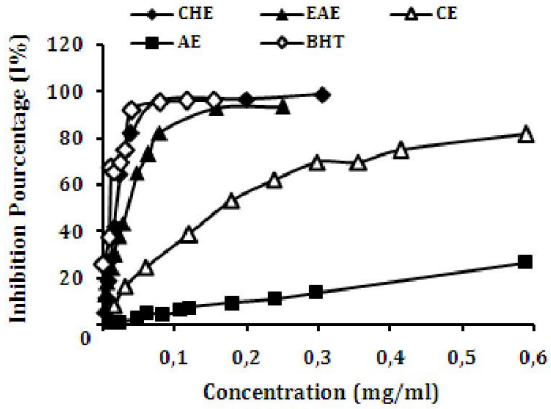

Fig. 1. Inhibition percentage of DPPH radical by TCRE fractions CHE: chloroform extract, EAE: ethyl acetate extract, CE: crud extract and AE: aqueous extract, Blank: BHT (butylated hydroxytoluene).

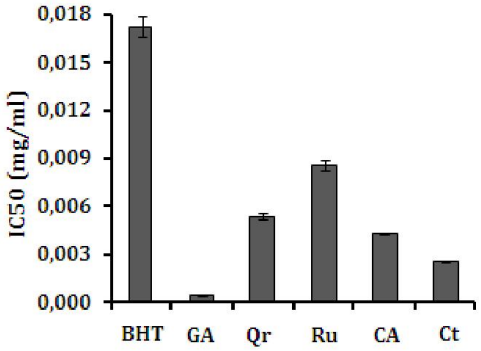

Fig. 2 Free radical scavenging capacities of pure polyphenols measured in DPPH assay. GA: Gallic acid, Qr: Quercetin, Ru: Rutin, CA: Cafeic acid and Ct: Catechin.

Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their color, and thus absorbance, for a longer time.

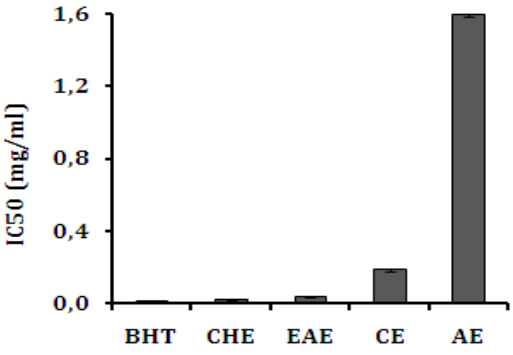

Fig. 3 Free radical scavenging capacities of the *Tamus communis* root extracts measured in DPPH assay. CHE: chloroform extract, EAE: ethyl acetate extract, CE: crud extract and AE: aqueous: extract. Blank: BHT (butylated hydroxytoluene).

In the β-carotene/linoleic acid assay, all pure compounds and TCRE showed marked antioxidant activities when compared to the synthetic antioxidant BHT which had 96% at the same concentration (2 mg/mL). The inhibition ratios of linoleic acid oxidation by pure polyphenols (Fig 4) were too close to each other (Rutine 95.36% ± 1.91, Catechine 93,38 % ± 1,49, Gallic acid 83,83 %± 2,85 and Cafeic acid 88,31% ± 2,29). TCRE have expressed an antioxidant power more or less comparable to those of BHT, especially non polar fractions (Fig 5). The antioxidant activity of TCRE followed the order of: CHE (89,838 % ± 2,04) > EAE (87,991 % ± 1,45) > CE (71,786 % ± 2,02) >>> AE (52,386 % ± 1,51). The higher radical-scavenging activity of TCRE especially CHE could be related to the presence of phenolics such as phenolic acids and flavonoids which play an important role in the radical scavenging activity (Baghiani *et al*., 2010).

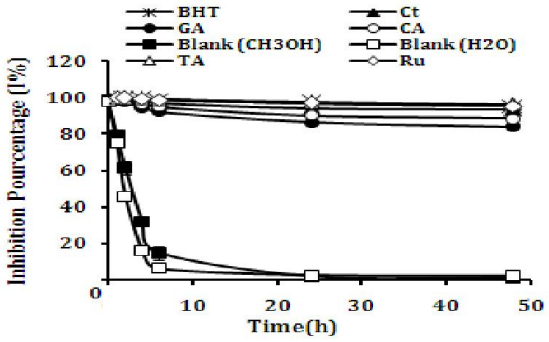

Fig. 4 Absorbance change of ß-carotene at 490 nm in the presence of pure polypheols (Rutin, Catechine, Gallic acid, Cafeeic acid, Tannic acid) and butylated hydroxytoluene (BHT).

Antibacterial activity, The application of various TCRE on the nine bacterial strains tested, showed that both fractions insoluble in water are the only ones that showed activity against several bacterial strains of both Gram positive and negative, since the CHE rich in flavonoids aglycones, has the spectrum of activity as broad as they exert an inhibitory action on growth of four bacterial strains, S. aureus, E. agglomerans, S. marcescens, and Bacillus sp were inhibited by CHE of T. communis with areas (14 mm, 14 mm, 12 mm and 10 mm respectively). Phytochemical analysis of plant extracts indicates that the presence of one or more groups of phytoconstituents like flavonoids, tannins, glycoside, phenols, saponin, etc. is responsible for antibacterial activity alone or in combinations (Iqbal and Farrukh, 2007).

Table 2. Total Polyphenol And Flavonoid Contents Of Tamus Communis Root Extracts (Tcre)

Extracts	Total Polyphenols Mg equivalent gallic acid g-1 lyophilisat	Total Flavonoids Mg equivalent quercetin g-1 lyophilisat
CE	24.756 ± 6.663	02.440 ± 0.440
CHE	78.222 ± 2.251	07.934 ± 0.993
EAE	61.432 ± 9.466	10.265 ± 4.891
AE	04.669 ± 0.616	01.287 ± 1.009

HE: chloroform extract, EAE: ethyl acetate extract,
CE: crud extract and AE: aqueous: extract.
Each value represents the mean ± SD (n = 3).

Flavonoids have been reported especially for moderate bacteriostatic properties (Xu and Lee, 2001). Tripoli *et al*., (Tripoli *et al*, 2007), found that the structure of flavonoids

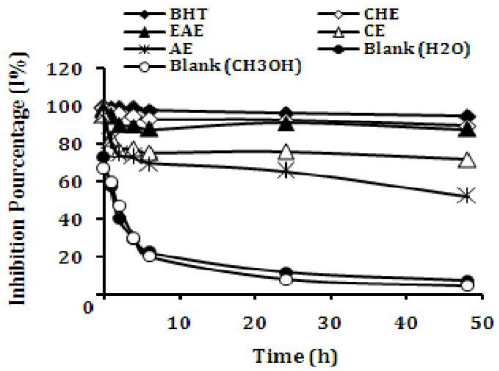

Fig. 5 Absorbance change of ß-carotene at 490 nm in the presence of *Tamus comminus L.* extracts (CHE: chloroform extract, EAE: ethyl acetate extract, CE: crud extract and AE: aqueous extract) and butylated hydroxytoluene (BHT).

used in combinational therapy. Such compounds or active fractions may not necessarily have strong antibacterial activity but may enhance the activity of classical antibiotics synergistically by above-known or novel mode of action (Iqbal and Farrukh, 2007).

Conclusion

Tamus comminus L. root extracts (TCRE) have significant antioxidant effect, free radical scavenging properties and moderate antibacterial activity due to their higher content of phenolic compounds especially flavonoids. The inter-relationship between antioxidant, antibacterial activities and phenolic compounds, is a promising to understand and to elucidate possible mechanisms for the functionality of traditional medicinal plants taken for diseases prevention and treatment.

Table 2. Antibacterial activity of Tamus communis root extract and their fractions, Zone of Inhibition (diameter in mm)

Strains	CHE	EAE	CE	AE	Rut	Quer	Gal. A.
S. aureus	14 ± 0,58	11± 1,00	-	-	-	-	20
Bacillus sp	10 ± 0,58	-	-	-	13	11	19
C. freundii	-	-	-	-	-	-	11
E.agglomerans	14 ± 1,53	10 ± 1,00	-	-	-	-	13
K. pneumonia	-	-	-	-	-	-	13
P. aeruginosa	-	-	-	-	11	10	12
E. coli	-	-	-	-	-	-	13
S. typhi	-	-	-	-	-	-	10
S. marcescens	12 ±0.58	09±0.58	-	-	-	-	10

CHE: chloroform extract, EAE: ethyl acetate extract, CE: crud extract and AE: aqueous extract, Rut : Rutin, Quer : Quercetin, Gal. A: Gallic acid.

Table 3. Antibiotic sensitivity of the bacterial strains tested, Zone of Inhibition (diameter in mm)

Strains	Nib (20 µg)	PB (300 UI)	Néo (30 UI)	Dot (30 UI)	Cmp (30 µg)	Gen (10 UI)	E (15 UI)	AMC (20/10 µg)	Pen (6 µg)	CZ (30 µg)	CTX (30µg)	Suf (200µg)
S. aureus	08	12	16	12	11	-	09	-	-	-	-	-
Bacillus sp	22	11	16	11	15	-	-	-	-	-	-	-
C. freundii	21	12	14	13	16	-	-	-	-	-	-	-
E.agglomerans	22	12	11	-	-	-	-	-	-	-	-	-
K. pneumonia	22	14	16	16	17	16	-	19	-	15	12	14
P. aeruginosa	-	13	-	-	-	11	-	-	-	-	-	-
E. coli	22	13	16	18	23	16	-	16	-	15	13	09
S. typhi	21	13	15	-	-	-	-	-	-	-	-	-
S. marcescens	20	12	15	09	15	16	10	-	-	-	-	-

Nib : Nibiol, PB : Polymyxin, Neo : Neomycin, Dot : Doxycylin, Cmp : Cloramphenicol, Gen : Gentamicin, E : Erythromycin, AMC: Amoxicillin + Clavulanic acid, Pen: Penicillin, CZ: Cefazolin, AMX: Amoxicillin, Lin: Lincomycine, Suf: Sulfamid Forts, CTX: Cefotaxim.

plays an important role in their activity, or flavonoids aglycones are more active against bacterial strains than glycosylated flavonoids. Several flavones polymethoxylated were found strongly inhibitory of bacterial lipopolysaccharide induces the expression of TNF-α, whereas glycosylated flavonoids were inactive (Manthey *et al*., 1999).

Novel antibacterial actions of plant extracts or phytocompounds have been documented which include inhibition of MDR-efflux pump (Stermitz *et al*., 2000) and ß-lactamase activity (Yam *et al*., 1998), anti-antibiotic resistance properties (Lee *et al*., 1998) and R-plasmid elimination (Beg and Ahmad, 2000). Similarly, few plants extracts and phytocompounds exhibited synergistic interaction with antibiotics against Gram-positive bacteria (Aqil *et al*., 2005). Screening of crude extracts for synergistic interaction with antibiotics is expected to provide bioactive compounds to be

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