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ORIGINAL ARTICLE

Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (*Astraceae*) and *Ziziphus lotus* (*Rhamnacea*)

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Abstract The present work quantitatively evaluates the antimicrobial and antioxidant potentials of two Libyan folk medicinal plants [*Artemisia campestris* (*Astraceae*) and *Ziziphus lotus* (*Rhamnacea*)] that commonly grow in the south of Libya. The crude methanolic leaves extracts of both plants are appreciably active against Gram-positive species, associated with week anti-Gram-negative activity. These two plant extracts also showed reasonably high contents of polyphenolics and alkaloids, with minimal inhibitory concentrations between 12.5–25 and 250–1000 μg/ml for Gram-positive and Gram-negative species, respectively. Results collectively suggest that *A. campestris* and *Z. lotus* are not only reliable natural sources of antimicrobials but also potential sources of phenolic antioxidants and hence could be nominated for future intensive studies.

1. Introduction

The Mediterranean climate in Libya favors the growth of a great number of plant species, some of which have various medicinal and antioxidant potential properties (Kotb, 1985; El-Naili et al., 2008). The use of plant extracts and phytochemicals, both with known reliable antimicrobial and antioxidant efficacies, can be of great significance in therapeutic approaches of many diseases (Lewis and Elvin-Lewis, 1997).

Over the years and up to date, there have been several studies documenting the antibacterial, antifungal, antiviral, anticancer and anti-inflammatory properties of plant ingredients (El-Naili et al., 2008; Harrison and Bartels, 2006; Benedek et al., 2007; Esra et al., 2007; Jebril et al., 2008). Therefore, herbal-derived substances remain the basis for large proportion of the commercial medications used today in developing countries for treatment of age-related brain diseases (Adams et al., 2007; Alencar et al., 2007; Orhan et al., 2007).

In fact, Libyan (Kotb, 1985) and some other Arab indigenous cultures (Saad et al., 2005) had a long standing

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tradition of healing with medicinal plants. When one refers to plants of medicinal value, one often lists their active ingredients, which might include alkaloids, flavonoids or glycosides (Luis et al., 2006), essential oils (Ekwenye, 2005), tannins (Dahanukar et al., 2000) and some other unusual substances.

Alkaloids are group of nitrogen-containing organic compounds; many of them are poisonous, whilst others are very useful as codeine and morphine which alleviate pain. Alkaloids (McDevitt et al., 1996) and essential oils (Vanida et al., 2005) are most often effective on skin and mucous membranes as expectorants, while yellow colored flavonoids, are found in many plants and can be used to treat atherosclerosis and hypertension (Luis et al., 2006).

Antioxidant activities of plant-origin polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations (Moosmann and Behl, 1999; Parr and Bolwell, 2000). Polyphenolic compounds in plants might also display distinctive anticarcinogenic, antimutagenic and cardioprotective effects linked to their free radical scavenging properties (Santos-Buelga and Scalbert, 2000; Alghazeer et al., 2008).

Polyphenols are also reported to be chemopreventive agents by lowering cholesterol and roughly limiting cell damage (Ferreira and Stade, 2002). These justify the overwhelming interest in finding new naturally occurring safe antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Parr and Bolwell, 2000).

The aim of this study is to evaluate the antimicrobial and antioxidant activities of *Artemisia campestris* (*Astraceae*) and *Ziziphus lotus* (*Rhamnaceae*) plants growing in south part of Libya. Both plants have not subjected before to detailed study to reveal their quantitative antimicrobial and anti-oxidant potentials. However, *A. campestris* has been mentioned and used in popular culture for centuries; it has several medicinal uses such as antispasmodic and antihelmintic characteristics (Kotb, 1985). *Z. lotus* is a deciduous shrub, native of the Mediterranean region (Kotb, 1985) and used to treat sore throats, alleviate stress and helps in the common colds.

2. Materials and methods

2.1. Plant materials

The plant materials (leaves) of *A. campestris* (family*: Astraceae*) and *Z. lotus* (family: *Rhamnaceae*) that utilized in this study were collected during the late flowering season (February–May, 2007) from the southern part of Libya. The herbarium specimens were authenticated by examining the morphological and anatomical features in the Botany Department, Faculty of Science Al-Fateh University, Tripoli, Libya.

2.2. Chemicals and reagents

All chemicals used during this work were products of Aldrich and Sigma Chemical Companies of reliable commercial grade, therefore utilized without further purification. Folin–Ciocalteu reagent (FCR) was always freshly prepared following the procedure described in literature (Varley et al., 1976). Silica gel 60 F254 thin chromatographic plates were purchased from Merck (Germany).

2.3. Bacterial strains

For assaying the antibacterial potential of the plant extracts, a narrow spectrum of antibiotics-sensitive clinically isolated bacterial species (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) was used. These organisms were generously provided by the Laboratory of Clinical Microbiology, Tripoli Medical Center, Tripoli, (Libya). Bacterial strains were routinely grown and preserved on Nutrient Broth or Mueller Hinton (NB, Difco) medium (2.0 % agar was added whenever needed). Antifungal testing of the extracts was out of concern in this work due to infection consideration.

2.4. Preparation of plant extracts

The fresh parts of both plants were washed enough with tap water then twice with distilled water, open air-dried for a week in shadowed place (26 ± 2.0 °C) then crushed into powder with an electric blender. Samples (10 g of the dried plant material) were individually soaked in 100 ml methanol and shaken for 24 h at room temperature (25 ± 2.0 °C), then filtered through sterile cotton till clear filtrate obtained.

2.5. Phytochemical tests

The fresh methanolic crude extracts were qualitatively screened (Harborne, 1983) for the following constituents: flavonoids, coumarines, hydrolysable tannins, alkaloids, terpenes, anthraquinones and saponins. The qualitative results of the methods have been rated from (+ve) for faint to (++++ve) for dense turbidity (Table 1).

2.5.1. Extraction of alkaloids

The strong positive result of alkaloids in both plants (*see results*) prompted us to adopt a special procedure (Hadi and Bremner, 2001) for individual extraction and testing for bioactive alkaloids. The summarized procedure for extraction of the alkaloids from *A. campestris* and *Z. lotus* is outlined (Fig. 1). Powdered plant materials were extracted in methanol with occasional swirling. Methanol extraction was continued until the plant material gave a negative result for alkaloids (Mayer's test). The obtained methanolic extract was evaporated under reduced pressure at 40 °C, to minimize any possible thermal degradation of the alkaloids and other thermolabile compounds. The crude alkaloid mixture was then separated from neutral and acidic materials, and water soluble ingredients, by initial extraction with aqueous acetic acid followed by dichloromethane extraction and then basification of the aqueous solution followed by further dichloromethane extraction.

2.5.2. Extraction of flavonoids

Also, the strong positive result of flavonoids in both plant extracts motivated us to perform an individual extraction and testing for flavonoids as described (Harborne, 1983). The plant tissues were hydrolyzed with HCl (2 M) for 30–40 min at 80 °C using water bath, then cooled to room temperature, the acidic solution was extracted twice with methanol and the combined extracts were evaporated to dryness. The crude residue was re-dissolved in the least volume of methanol for flavonoids

Table 1 Phytochemical screening of *Artemisia campestris* and *Ziziphus lotus*.

Phytochemical compounds	*Artemisia campestris* (*Astraceae*)	*Ziziphus lotus* (*Rhamnacea*)
Alkaloids	+++ve	++ve
Saponins	+++ve	++ve
Anthraquinones	−ve	−ve
Tannins	−ve	++ve
Terpenes	++ve	++ve
Flavonoids	+++ve	++ve
Coumarines	−ve	−ve

testing; then bioassays and thin layer chromatography were conducted.

2.5.2.1. In vitro antimicrobial assay and MIC determination Growth inhibition activities of methanolic leaves extract of *A. campestris* and *Z. lotus* against Gram-Positive and Gram-negative bacterial species (Table 2) were tested using the conventional paper disc assay (Bauer et al., 1966). A loop of bacterium from stock agar slant cultures was cultured in nutrient broth overnight (at 37 °C) and spread with a dry sterile cotton swap onto triplicate set of Petri plates each containing 25 ml of sterile solidified Mueller Hinton agar medium. Twenty four hours post incubation (at 37 °C ± 1.0); diameters of inhibition zones (DIZs) induced by extract-loaded discs (12.5–1000 μg/disc) were measured and approximated.

Table 3 The *in vitro* MIC values of methanolic crude extracts of *A. campestris* and *Z. lotus*.

Test organism	*A. campestris*	*Z. lotus*
Bacillus subtilis	12.5	12.5
Staphylococcus aureus	12.5	25.0
Escherichia coli	250.0	1000
Pseudomonas aeruginosa	500	1000
Salmonella typhi	250.0	1000

The minimal inhibitory concentrations (MICs) values of both plant extracts were assessed (Jorgensen et al., 1995) as shown in Table 3. The MIC was defined as the lowest concentration (μg/ml) of the tested material that completely inhibited the growth of the test organism. For bioautographic assay (Nostro et al., 2000) 10 μl of each extract (90 mg/ml) were applied onto 5 × 20 cm Silica gel 60 PF254 glass plate. Best separation was accomplished using Petroleum ether: Ethyl acetate (3:1) for both extracts (Fig. 2). The developed TLC plates were dried under a continuous stream of air for 3 h till complete removal of the solvent. The dried chromatograms were overlaid with nutrient agar medium seeded with *B. subtilis* (10^6–10^7 cfu/ml) and then incubated for overnight at 37 °C, Fig. 2. For comparative purposes, additional chromatogram was run

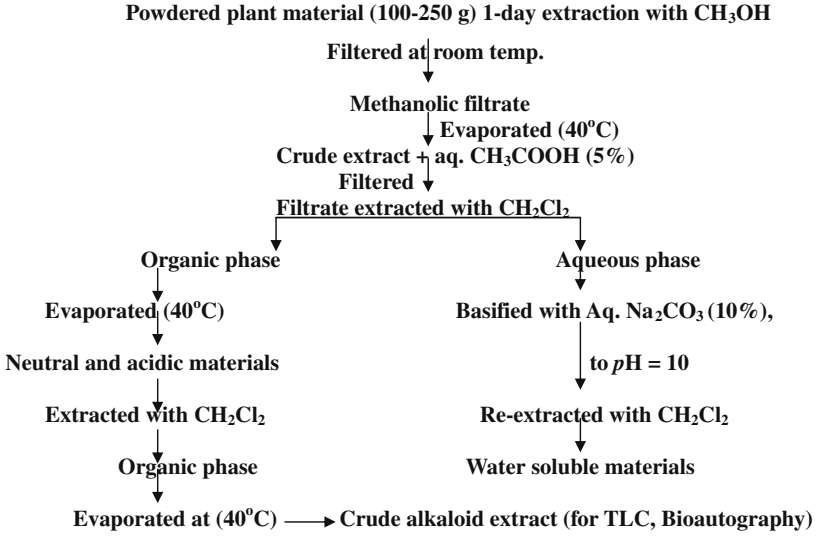

Figure 1 Outlines of the extraction procedure for alkaloids from *A. campestris* and *Z. lotus*.

Table 2 *In vitro* antibacterial activity of crude methanolic extracts of *Artemisia campestris* and *Ziziphus lotus*.

Test organism	*A. campestris*	*Z. lotus*	Tetracycline 30 μg/disc	Ceftazidime 30 μg/disc
Bacillus subtilis	32.0	29.0	25.0	27.0
Staphylococcus aureus	27.0	18.0	26.0	26.0
Escherichia coli	10.0	7.0	11.0	10.0
Pseudomonas aeruginosa	9.0	8.0	NT	NT
Salmonella typhi	8.0	7.0	10.0	12.0

DIZ, diameter of inhibition zone (NB. paper disc diameter = 5 mm) NT, not tested.

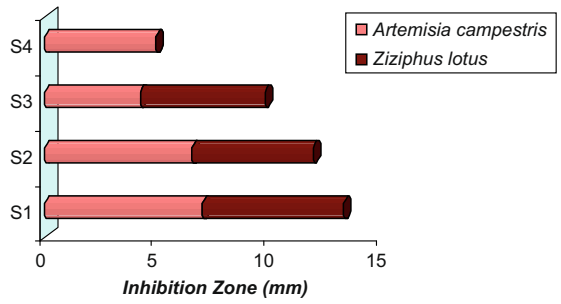

Figure 2 Bioautography of the TLC-fractionated crude methanolicextracts of *A. campestris* and *Z. lotus* against *B. subtilis*.

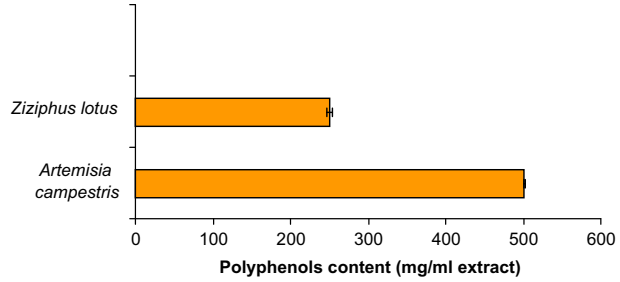

Figure 3 Total polyphenols content of *A. campestris* and *Z. lotus*. (Results represent the mean of triplicate experiments).

alongside under same conditions and sprayed for visualization of alkaloids and flavonoids (Krebs et al., 1969).

2.5.3. Determination of total phenolics

Total phenolics were assessed by the Folin–Ciocalteu method (Singleton et al., 1999). A volume of 20 µl sample (0.1 g/ml) was mixed with 1.5 ml distilled water and then 100 µl of diluted Folin–Ciocalteu reagent (1:2 v/v, in distilled water). After that, 300 µl of 20% sodium carbonate were added. The final mixture was shaken thoroughly and incubated at room temperature in dark place for 2 h.

Thereafter, the absorbance of the samples was measured at 765 nm. Gallic acid (0–500 mg/l) was used for calibration of a standard curve. The results are expressed as gallic acid equivalents (GAE)/g dry weight of the plant tissue. Triplicate measurements were taken and the mean values were calculated and statistically analyzed (Fig. 3).

3. Results and discussion

A. campestris (family*: Astracea*) and *Z. lotus* (family: *Rhamnacea*) are morphologically described in the encyclopedia of Libyan flora (Kotb, 1985). It has several medicinal uses such as antispasmodic and antihelmintic characteristics.

Z. lotus is a deciduous shrub, native of the Mediterranean and other region. It is used to treat sore throats, alleviate stress and helps in the common colds (Harrison and Bartels, 2006; Saad et al., 2005). The reported antimicrobial activities of different species related to *Astracea* and *Rhamnacea* (Harrison and Bartels, 2006) are mainly attributed to its most active ingredients, polyphenols and alkaloids. This prompted us to subject these two species, that grow in Libya, for extraction and identification of their phytochemicals with possible antimicrobial and antioxidant activities.

3.1. Extraction of bioactive substances

Although water is almost universally shown as the practical solvent used to extract activity, the current results revealed a reasonable existence of different ingredients extracted in methanol, especially from leaves. Whilst aqueous extracts of these parts are shown of very weak or lacking inhibitory effects against test bacteria (results are not shown) (Table 1). This might indicate the relative impracticability of water (at 25 ± 2 °C) as extracting solvent for the active antimicrobial compounds of these plants. In this regard, many reports declared that organic solvents (used in single or mixed forms) especially polar ones are most preferable for extraction of biologically active plant ingredients (De Pasquale et al., 1995; Ferrero et al., 2007).

Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanolic or methanolic extraction (Nostro et al., 2000). Methanol and ethanol are reported as efficient extracting solvents for saponins and sterols (De Pasquale et al., 1995; Hui et al., 2007), alkaloids (Ivanovska et al., 1996), polyphenols (Ferrero et al., 2007) terpenoids (Taylor et al., 1996) while dichloromethane is efficient in terpenoids extraction (Mendoza et al., 1997; Cowan, 1999).

Therefore, initial screenings of plants for possible antimicrobial activities typically begin by using crude alcohol extractions and can be followed by various organic extraction methods. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides and polypeptides, including fabatin (Zhang and Lewis, 1997) and various lectins, are commonly more effective as antiviral, however, are not intended to be identified in the antibacterial screening techniques used in the current study. Occasionally few tannins and terpenoids are found in the aqueous phase of some plants, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

In the present study, leaves of these two species , tested negative for anthraquinones and coumarines, while positive for flavonoids (+ + + ve), tannins (+ + ve only in *Z. lotus*) which was in parallel with polyphenolics content results (Fig. 3), saponins (+ + + ve) with strong positive results indicating their richness of alkaloids (+ + + + ve) and moderate for terpenes (+ + ve) (Table 1).

Saponins, alkaloids are reported as the most active ingredients to which the antimicrobial activities of many plant species are attributed (Harrison and Bartels, 2006). This prompted us to choose these plants for further investigations focusing on their biologically active ingredients. Although leaves and barks of different plant species are shown to harbor antibacterial alkaloids, many other plant roots or rhizomes are also reported to possess high (+ + + ve) to moderate (+ + ve) alkaloid content (Hadi and Bremner, 2001). Some of these species are *Curcuma xanthorrhiza* (+ + + ve); *Sterculia foetida* (+ + + ve); *Oleifera Lamk* (+ + ve); *Ficus septica* (+ + + ve); *Alstonia cholaris R.Br.* (+ + + ve); *Strychnos ligustrina B.* (+ + ve); *Michelia champaca L.* (+ + ve) and therefore reasonably used

for treatments of fevers, diarrhea, skin diseases, abscesses and wounds in African folk medicine (Hadi and Bremner, 2001; Edeoga et al., 2005). The alkaloid components of the orange-colored latex of *Michelia champaca L.* (chelidonine, chelerythrine, coptisine, sanguinarine, and berberine) (Then et al., 2000) were found to have significant biological and pharmacological activities such as spasmolytic, anti-inflammatory, antimicrobial, antiviral, antifungal and antitumor, besides cytotoxic properties (Khayyal et al., 2001; Coon and Ernst, 2002; Kokoska et al., 2002).

After initial screening of phytochemicals, more detailed investigation of their antagonistic effects is recommended to be conducted. Therefore, and at this stage in the current study, more specific medium was used and the antimicrobial activity was compared to two of currently used antibiotics of broader spectrum, namely Tetracycline and Ceftazidime (Table 2).

Artemisia and *Ziziphus* methanolic extracts showed good inhibitory effects only against Gram-positive with no antagonistic effects against Gram-negative bacterial species tested (Table 2). The MIC values of the crude methanolic *A. campestris* and *Z. lotus* extracts was on the range 12.5 and 25.0 μg/ml on Gram-positive, whereas both extracts exhibited very weak anti microbial activity against Gram-negative with very high values (250–1000 μg/ml) of MICs (Table 3).

Mode of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, it was found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity (Mendoza et al., 1997). The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *B. subtilis* and *S. aureus* and lesser activity against Gram-negative bacteria as well as *Candida albicans (*Hufford et al., 1993). On the other hand, two isolated diterpenes (Batista et al., 1994) were found to be more efficient against *S. aureus*, *V. cholerae*, *P. aeruginosa*, and *Candida* spp.

Since initial screening of potential antibacterial compounds from plant materials may be equivocally performed either with pure substances (Batista et al., 1994; Klopoukh et al., 1997) or crude extracts (Freiburghaus et al., 1996; Silva et al., 1996), we adopted the two most commonly used procedures of broth dilution assay (Hess et al., 1995) to determine antimicrobial susceptibility of crude extracts, while the two-fold dilution assay (Navarro et al., 1996) was used for MIC determination. Adaptations such as the agar overlay method (Bioautography) (Nostro et al., 2000) were also made to specify the biologically active spots (against *B. subtilis*) on TL Chromatograms from both plants (Table 4).

Thin Layer-bioautographic application revealed the presence of four biologically active spots (S_1–S_4) with R_f values of 0.15–0.69 from *Artemisia* and of 0.14–0.67 from *Ziziphus*, all were positive with alkaloids spray reagent. Same pattern of flavonoids separation was also obtained from fractionated crude extracts when subjected to bioautography. Also four biologically active spots are allocated with R_f values of 0.20–0.72. The two plant extracts seem to possess few alkaloid spots (S_1 and S_4) in common (with R_f values, 0.14, and 0.15, respectively) and flavonoids (S_4) indicating their relevance in chemical nature.

The investigation of plant extracts effective against methicillin-susceptible and resistant *S. aureus* (Tsuchiya et al., 1996; Sato et al., 1997) provides an example for prospecting for new compounds which may be particularly effective against clinical infections that are currently difficult to treat. The activity of three extracts from *Terminalia chebula* RETS were examined against methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA) as well as 12 other Gram-negative and Gram-positive bacteria (Sato et al., 1997). Diterpenoid alkaloids, commonly isolated from the plants of the *Ranunculaceae*, or buttercup family, are commonly found to have distinctive antimicrobial properties (Atta-ur-Rahman and Chaoudhary, 1995; Omulokoli et al., 1997). Solamargine, a glycoalkaloid from the berries of Solanum khasianum, and other alkaloids were found useful against HIV infection (Sethi, 1979; McMahon et al., 1995) as well as intestinal infections associated with AIDS (McDevitt et al., 1996). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987).

It was found that extracts containing gallic acid derivatives were more effective against both types of *S. aureus* than they were against other species (Sato et al., 1997). Actually, the effect of both crude and fractionated extracts of each plant against resistant *S. aureus* clinical isolates will be the subject of intensified study to disclose the reliability to treat threatening infections caused by species like MRSA.

Although the mechanism of action of terpenes is not fully understood, nonetheless, is speculated to involve membrane disruption by the lipophilic compounds (Mendoza et al., 1997).

Laboratories of the world have found literally thousands of phytochemicals of pronounced *in vitro* inhibitory effects on all types of microorganisms. Alkaloids from *A. campestris* and *Z. lotus* might be added to the list. More of these compounds including other phytochemicals should accept more attention in Libya to be subjected to animal and human trial studies to determine their effectiveness in whole-organism systems, including in particular, toxicity studies as well as an examination of their effects on beneficial normal microbiota. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infections using plant-derived principles and drug design programs.

Table 4 Bioautographic profile[b] (with R_f values) of alkaloid and flavonoid fractions in *A. campestris* and *Z. lotus*.

Spot No.	Alkaloid fractions[a]				Flavonoid fractions[a]			
	S1	S2	S3	S4	S1	S2	S3	S4
A. campestris	0.15	0.31	0.48	0.69	0.30	0.43	–	0.70
Z. lotus	0.14	0.19	0.43	0.67	0.20	0.28	0.39	0.72

[a] All spots separated are active against the most sensitive test bacterium (*B. subtilis*).
[b] Best separation was accomplished using petroleum ether:ethyl acetate (3:1) for both extracts.

4. Conclusion

A. campestris and *Z. lutos*, being local medicinal plants with great abundance in south of Libya are shown rich in anti-Gram-positive phytochemicals including alkaloids in addition to their considerable content of antioxidant flavonoids. These results give them the privilege to start intensive detailed analytical studies for isolation and identification of these individual biologically active ingredients for local drug design programs in qualified and interested Pharmacological Centers. Wider spectrum of test species including MRSA, fungi and viruses is also recommended to be used for re-evaluation of these extracts.

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References

Adams, M., Gmunder, F., Hamburger, M., 2007. J. Ethnopharmacol. 119, 203.
Alencar, S.M., Oldoni, T.L.C., Castro, M.L., Cabral, I.S.R., Costa-Nero, C.M., Curry, J.A., Rosalin, P.L., Ikigaki, M., 2007. J. Ethnopharmacol. 114, 93.
Alghazeer, R., Gao, H., Howell, N.K., 2008. Toxicol. Lett. 180, 202–211.
Atta-ur-Rahman, R., Chaoudhary, M.I., 1995. Nat. Prod. Rep. 12, 361.
Batista, O., Duarte, A., Nascimento, J., Simones, M.F., 1994. J. Nat. Prod. 57, 858.
Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Am. J. Clin. Pathol. 45, 493.
Benedek, B., Kopp, B., Melzig, M.F., 2007. J. Ethnopharmacol. 113 (3), 384.
Coon, J.T., Ernst, E., 2002. Aliment. Pharm. Ther. 16, 1689.
Cowan, M.M., 1999. Clin. Microbiol. Rev. 12 (4), 564.
Dahanukar, S.A., Kulkarni, R.A., Rege, N.N., 2000. Indian J. Pharmacol. 32, S81–S118.
De Pasquale, R., Germano, M.P., Keita, A., Sanogo, R., Lauk, L., 1995. J. Ethnopharmacol. 7, 55.
Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., 2005. African J. Biotechnol. 4, 685.
Ekwenye, U.N., 2005. J. Ethnopharmacol. 111, 97.
El-Naili, M.A., Saleh, N.A., Ahmed, H.I., Rammash, B.K., El-Buni, A.A., 2008. J. Egypt Acad. Soc. Environ. Develop. 9, 1.
Esra, K., Tuson, A., Yesilada, E., 2007. J. Ethnopharmacol. 113, 332–337.
Ferreira, D., Stade, D., 2002. Nat. Prod. Rep. 19, 517.
Ferrero, A., Menitti, A., Bras, C., Zanetti, N., 2007. J. Ethnopharmacol. 113, 78.
Freiburghaus, F., Kaminsky, R., Nkunya, M.H.H., Brun, R., 1996. J. Ethnopharmacol. 55, 1.
Hadi, S., Bremner, J.B., 2001. Molecules 6, 117.
Harborne, J.B., 1983. Phytochemical Methods. Chapman and Hall, London, p. 288.
Harrison, A.P., Bartels, E.M., 2006. Am. J. Pharm. Toxicol. 1 (2), 26.
Hess, S.C., Brum, R.L., Honda, N.K., Cruz, A.B., Moretto, E., Cruz, R.B., Messana, I., Ferrari, F., Filho, V.C., Yunes, R.A., 1995. J. Ethnopharmacol. 47, 97.
Hufford, C.D., Jia, Y., Croom Jr., E.M., Muhammad, I., Okunade, A.L., Clark, A.M., Rogers, R.D., 1993. J. Nat. Prod. 56, 1878.
Hui, M.E., Cheng, E.H., Radhakrishnan, A.K., 2007. J. Ethnopharmacol. 110 (3), 406.
Ivanovska, N., Philipov, S., Istatkova, R., Georgieva, P., 1996. J. Ethnopharmacol. 54, 143.
Jebril, A.O., 2008. A Screening Study on the Antimicrobial and Hepatoprotective Effects of Some Medicinal Plants in Libya. MSc. Thesis, Faculty of Science, Al-Fateh University, Tripoli, Libya.
Jorgensen, J.H., Swenson, J.M., Tenover, F.C., Barry, A., Ferraro, M.J., Murray, P.R., Reller, B., 1995. J. Clin. Microb. 34, 2679.
Khayyal, M.T., El-Ghazaly, M.A., Kenawy, S.A., Seif-El-Nasr, M., Mahran, L.G., Kafafi, Y.A.H., Okpanyi, S.N., 2001. Arzneimittel-Forsch 51, 545.
Klopoukh, L., Siddiqi, S., Warns, M., To, L., 1997. In Abstracts of the 97th General Meeting of the American Society for Microbiology. American Society for Microbiology, Washington, DC. A-199.
Kokoska, L., Polesny, Z., Rada, V., Nepovim, A., Vanek, T., 2002. J. Ethnopharmacol. 82, 51.
Kotb, H.T.F., 1985. Medicinal Plants in Libya, Part II.
Krebs, K.G., Heusser, D., Wimmer, H., 1969. In: Stahl, E. (Ed.), Handbook. Springer-Verlag, Berlin, pp. 854–905.
Lewis, W.H., Elvin-Lewis, M.P., 1997. Ann. Mol. Bot. Gard. 82, 16.
Luis, J.C., Valdés, F., Martín, R., Carmona, A.J., Díaz, J.G., 2006. Int. J. Mol. Med. Adv. Sci. 1, 411.
McDevitt, J.T., Schneider, D.M., Katiyar, S.K., Edlind, T.D., 1996. In: Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC (Abstract 175).
McMahon, J.B., Currens, M.J., Gulakowski, R.J., Buckheit, R.W.J., Lackman-Smith, C., Hallock, Y.F., Boyd, M.R., 1995. Antimicrob. Agents Chemother. 39, 484.
Mendoza, L., Wilkens, M., Urzua, A., 1997. J. Ethnopharmacol. 58, 85.
Moosmann, B., Behl, C., 1999. Proc. Natl. Acad. Sci. USA 96, 8867.
Navarro, V., Villarreal, M.L., Rojas, G., Lozoya, X., 1996. J. Ethnopharmacol. 53, 143.
Nostro, A.M.P., Germano, Á., D'Angelo, V., Marino, A., Cannatelli, M.A., 2000. Lett. Appl. Microbiol. 30, 379.
Omulokoli, E., Khan, B., Chhabra, S.C., 1997. J. Ethnopharmacol. 56, 133.
Orhan, I., Kupeli, E., Terzioglu, S., Yesilada, E., 2007. J. Ethnopharmacol. 14, 32–37.
Parr, A.J., Bolwell, G.P., 2000. J. Sci. Food Agric. 80, 985.
Phillipson, J.D., O'Neill, M.J., 1987. Acta Pharm. Nord. 1, 131.
Saad, B., Azaizeh, H., Said, O., 2005. A Review. CAM, 1.
Santos-Buelga, C., Scalbert, A., 2000. J. Sci. Food Agric. 80, 1094.
Sato, Y., Odetani, H., Singyouchi, K., Ohtsubo, T., Kihara, M., Shibata, H., Higuti, T., 1997. Biol. Pharm. Bull. 20, 401.
Sethi, M.L.J., 1979. Nat. Prod. 42, 187–196.
Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A., Gomes, E., 1996. J. Ethnopharmacol. 50, 55.
Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Method Enzymol. 299, 152.
Taylor, R.S.L., Edel, F., Manandhar, N.P., Towers, G.H.N., 1996. J. Ethnopharmacol. 50, 97.
Then, M., Szentmihályi, K., Sárközi, A., Illés, V., Forgács, E., 2000. J. Chromatogr. Anal. 889, 69.
Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T., Iinuma, M., 1996. J. Ethnopharmacol. 50, 27.
Vanida, C., Somporn, P., Wiratda, W., 2005. J. Sci. Technol. 27, 813–818.
Varley, H., 1976. Determination of alkaline phosphatase. In: Varley, H. (Ed.), Practical Clinical Biochemistry, fourth ed. p. 453.
Zhang, Y., Lewis, K., 1997. Microbiol. Lett. 149, 59.