Tropical Journal of Pharmaceutical Research October 2012; 11 (5): 739-746
© Pharmacotherapy Group,
Faculty of Pharmacy, University of Benin,
Benin City, 300001 Nigeria.

All rights reserved.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v11i5.6

Research Article

Composition of the Essential Oil of *Clausena Suffruticosa* Leaf and Evaluation of its Antimicrobial and Cytotoxic Activities

Md. Atiar Rahman[1,3*] Joti Sankhar Chakma[1], Nazrul Islam Bhuiyan[2] and Md. Shahidul Islam[3]

[1, 2]Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, [2]Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong-4220, Bangladesh, [3]Department of Biochemistry, School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa.

Abstract

Purpose: *To investigate the essential oil content of Clausena suffruticosa leaf for its in-vitro antibacterial, antifungal and cytotoxic activities.*
Methods: *The essential oil of Clausena suffruticosa leaf was extracted by hydrodistillation using a modified Clevenger-type apparatus and was analyzed by GC-MS using electron impact ionization method. Antibacterial, antifungal and cytotoxic screenings were made by disc diffusion technique, poisoned food technique and brine shrimp lethality bioassay, respectively. Minimum inhibitory concentration (MIC) of the oil was determined by measuring the zone of inhibition, with tetracycline as reference standard. Fluconazole served as standard in the antifungal assessment.*
Results: *A total of twenty two compounds, of which Estragole, Anethole and β-Ocimene were the major ones, were found in the essential oil of C. suffruticosa. The oil showed higher antibacterial activity against Shigella flexneri than the reference, tetracycline ($p < 0.05$). Significant activity ($p < 0.001$) against other Gram-positive microbes - Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus polymyxa and Bacillus megaterium – was also observed. However, Gram-negative bacteria - Salmonella typhi, Shigella flexneri, Proteus mirabilis and Escherichia coli. Klebsiella pneumoniae and Shigella sonnei - showed no sensitivity to the oil. In the antifungal assay, the oil exhibited greater activity ($p < 0.001$) against Aspergillus ochraceus than the reference, fluconazole, Inhibition of other fungal strains tested was also statistically significant ($p < 0.001$). The lethal concentration (LC_{50}) of the oil against brine shrimp was 41.2 µg/ml in the cytotoxic assay.*
Conclusion: *It is evident that the essential oil of C. suffruticosa is a potent antimicrobial and cytotoxic agent that should be further evaluated.*

Keywords: *Clausena suffruticosa, Essential oil, Cytotoxicity, Antimicrobial, Brine shrimp*

Received: 31 January 2012 *Revised accepted: 11 August 2012*

*Corresponding author: ***Email:*** 211560713@ukzn.ac.za, atiarh@yahoo.com; **Tel:** +27 31 260 8362; **Fax:** +27 31 260 7942

INTRODUCTION

Clausena suffruticosa (Rutaceae), locally known in Chittagong, Bangladesh as Sadi Urisha or Kalomoricha, is a shrub-type plant and sparsely distributed in the hilly areas of Khagrachari and Sylhet in Bangladesh [1]. The plant is seldom found in Eastern Himalayan regions, Kashi Hill (India) and Burma. It has been commonly used as a herbal remedy by Chakma tribe in Bangladesh. Traditionally, the roots, leaves and seeds of the plant have been used for some chronic diseases such as paralysis, tumors, as well as diseases of the kidney and liver. It has also been used traditionally for the treatment of mumps, viral pneumonia, cerebrospinal meningitis, pain, bleeding and fever [2]. However, only limited scientific investigation has been carried out on the therapeutic properties of this plant [2].

As a part of our continuing investigations of the biological activities of Bangladeshi Rutaceae plants, the purpose of this study was to evaluate the composition of *C. suffruticosa* leaf essential oil, as well as its antimicrobial and cytotoxic activities.

EXPERIMENTAL

Collection and identification of plant material

The plant material was collected from Khagrachari in the Chittagong Hill tracts of Bangladesh during the month of June to July 2010. The plant was taxonomically identified by Dr Md Mostafa Kamal Pasha (Professor and taxonomist, Department of Botany, University of Chittagong, Bangladesh). A voucher specimen was preserved in Bangladesh National Herbarium (Accession no. DACD- 32909).

Extraction of essential oil

The fresh leaves (5 kg) of the healthy, well grown plant (2 - 3 years old) were soaked in distilled water and then hydrodistilled in a modified Clevenger-type apparatus (model 553, Hospital Equipment Mfg Co, India). Distillation of the materials was carried out for 6 h. The oil collected (2.5ml) was then dried over anhydrous sodium sulfate in a desiccator, stored in a sealed container and placed in a refrigeration at 4 ºC prior to analysis.

Gas chromatography-Mass spectrometric (GC-MS) analysis

The essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) (based on electron impact ionization (EI) method) consisting of GC-17A gas chromatograph (Shimadzu Corporation, Japan) coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu, Japan); fused silica capillary column (30 m x 2.5 mm, 0.25mm film thickness) coated with DB-1 (J&W); column temperature 100 °C (2 min) to 250 °C at the rate of 3 °C/min; carrier gas (helium) at constant pressure of 90 kPa. Acquisition parameters were at full scan while scan range was 40 – 350 amu. The composition of the oil was arrived at by comparing with mass spectra from NIST (Maryland, USA) Library (NIST 147 and NIST 27) [3,4].

Experimental media

Müeller-Hinton agar medium (Hi-media, India, final pH 7.3 ± 0.2, 25 °C) was used for the determination of antibacterial activity and minimum inhibitory concentration (MIC). On the other hand, potato dextrose agar media (Hi-media, India, final pH 5.6 ± 0.2, 25 °C) and artificial seawater (3.8 % NaCl solution) were used for antifungal and cytotoxic activity determinations, respectively.

Antibacterial test

In vitro antibacterial screening was carried out by qualitative to semi-qualitative disc diffusion method. Bacterial suspension turbidity was adjusted to McFarland standard number 0.5 in Mueller Hinton broth (Hi-media, India). With a sterile cotton swab, the bacterial culture medium was streaked on

previously prepared Mueller Hinton Agar plate (Hi-media, India). Dried and sterilized paper discs were treated separately with the desired concentration of previously prepared methanol solution of the essential oil (6 and 12 µl/disc) using a micropipette dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The plates were kept for 4 - 6 h at low temperature and the oil diffuse from the disc to the surrounding medium. The plates were then incubated at 37 °C for 18 h. The diameter of the zone of inhibition produced was compared with that produced by reference standard (positive control), tetracycline (30 µg/disc). Each determination was carried out in triplicate. Blanks disc impregnated with methanol was used as negative control.

Determination of minimum inhibitory concentration (MIC)

Serial dilution technique [5] was used to determine the MIC of the oil against tested organisms. Briefly, serial dilution of the essential oil was done using 10% Tween 80 in sterile nutrient broth. The 0.5 mL of the essential oil was added to 2 mL of the media and double fold serial dilution was carried out to give a concentration of 1, 2, 5, 10 and 40 µg /mL. Each strain of test organism was tested with essential oil by inoculating with 50 mL physiological saline containing $5x10^6$ bacterial cells. They were incubated at 37°C for 18-24 h. MIC was determined as described above for antibacterial assay using the reference antibiotic tetracycline. Results were recorded in millimeter after measuring the diameter of zone of inhibition

Antifungal test

The poisoned food technique [6] was used to screen for anti-fungal activity. Potato dextrose agar (PDA, acumedia, Neogen Corporation, Lansing, Michigan, USA) was used as the culture medium. The required concentration (10 and 16 µg/disc) volume and concentration of the oil was placed in a sterilized petriplate Petri plate, 15 ml of the culture medium was poured into it, mixed well and then allowed to solidify. Inoculation was done at the center of each plate with 5 mm mycelium block for the fungal strains *Aspergillus ustus*, *Aspergillus niger* and *Aspergillus ochraceus.* The mycelium block was prepared with the aid of a cork-borer from the growing area of a 5 day-old culture of the test fungus on PDA. The blocks were placed at the center of each Petri dish in an inverted position to achieve greater contact of the mycelium with the culture medium, and the inoculated plates were incubated at 25 ± 2 °C. The experiment was carried out in triplicate. The control (i.e., PDA without extract) was also tested. The diameter of the fungal colonies were measured after 5 days of incubation. The inhibition (%) of mycelial growth of the test fungus was calculated by the formula, I= (C-T)/C × 100, where I is inhibition (%), C the diameter of the fungal colony for control and T the diameter of the fungal colony for the test oil. Antifungal effect of the essential oil was compared with that of reference antifungal drug fluconazole (40 µg/disc, kindly donated by GlaxoSmithKline, Bangladesh).

Cytotoxicity test

The cytotoxic activity of the essential oil was determined by brine-shrimp lethality assay, as described by Meyer *et al* [7]. A simple zoological organism (*A. salina*) was used as the monitor for the test. The eggs of the brine shrimp were collected from Institute of Marine Science and Fisheries, University of Chittagong, Bangladesh, hatched in artificial seawater (3.8 % NaCl solution), and allowed to mature for 48 h to shrimp called nauplii. The essential oil test sample was prepared by dissolving 21 µl (10 mg/ml) of the oil dissolved in 2 ml of dimethyl sulfoxide (DMSO), from these 200, 100, 80, 40, 20 and 10 µg/ml solutions were prepared by serial dilution. Each concentration was tested in triplicate. A vial containing 50 µl DMSO diluted to 50 ml was used as control. Standard vincristine sulfate was used as reference (positive control). The shrimps, which were counted, were applied to each of

the experimental vials as well as the control vials. The number of nauplii that died after 24 h was counted. The LC_{50} values were calculated from probit Chart using software, Biostat®-2009 (AnalystSoft Inc. USA).

Statistical analysis

All data are presented as mean ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) using SPSS for Windows, version 18.0 (IBM Corporation, NY, USA). The values were considered significantly different at $p < 0.05$.

RESULTS

Composition of essential oil of *C. suffruticosa*

Several compounds were belonging to the classes of aldehydes, alcohols, terpenes, esters, acids and hydrocarbons were found in the oil. The major constituents were estragole, anethole and β-ocimene. The composition of the essential oil is shown in Table 1.

Antibacterial activity

The results of the antibacterial test are presented in Table 2.

Table 1: Composition of *C. suffruticosa* leaf essential oil

Constituent	Content (%)
α-Pinene	0.19
Sabinene	0.03
β-Myrcene	0.03
4-Hexen-1-ol, acetate	0.02
Limonene	0.02
β-Ocimene	1.40
1-Methylhexyl acetate	0.03
Linalol	3.38
2-Dodecen-4-yne, (E)-	0.02
Estragole	58.23
Linalyl acetate	0.06
Anethole	33.20
γ-Amylbutyrolactone	0.38
Eugenol	0.69
Caryophyllene	0.87
α-Caryophyllene	0.10
Isohomogenol	0.08
γ-Elemene	0.30
.+/-.-trans-Nerolidol	0.13
δ-Undecalactone	0.17
tau.-Cadinol	0.07
Cubenol	0.05

The oil showed a greater zone of inhibition (ZOI, 9.9 mm) against *S. flexneri* than tetracycline (9.0 mm). Antibacterial activity against Gram-positive (*B. cereus,* ZOI 15.4 mm; *B. subtilis,* ZOI 14.6 mm) and Gram-negative (*S. typhi*, ZOI 14 mm) bacteria was

Table 2: *In-vitro* antibacterial activity (mean ± SD, n = 3) of the essential oil of *C. suffruticosa* leaf

Test organism and type		Source ID	Zone of inhibition (diameter in mm)		
			Essential oil		Tetracycline (30µg/disc)
			(6µl/disc)	(12µl/disc)	
Gram-positive	*Bacillus subtilis*	BTCC 17	$10.5\pm0.14^{a***}$	$14.6\pm0.17^{b***}$	$22.0\pm0.57^{c***}$
	Staphylococcus aureus	BTCC 43	$7.4\pm0.15^{a**}$	$10.2\pm0.18^{b***}$	$24.0\pm0.57^{c***}$
	Bacillus cereus	BTCC 19	$11.3\pm0.15^{a***}$	$15.4\pm0.18^{b***}$	$18.1\pm0.08^{c***}$
	Bacillus polymyxa.	BTCC 16	$9.6\pm0.20^{a***}$	$12.6\pm0.29^{b***}$	$21.2\pm0.15^{c***}$
	B.megaterium	BTCC 18	$10.2\pm0.11^{a***}$	$13.1\pm0.88^{b***}$	$28.0\pm0.28^{c***}$
Gram-negative	*Klebsiella penumoniae*	ICDDR'B	-	-	$12.0\pm0.11^{c***}$
	Salmonella typhi	ICDDR'B	$11.0\pm0.15^{a***}$	$14.0\pm0.06^{b***}$	$27.0\pm0.08^{c***}$
	Shigella flexneri	ICDDR'B	$7.2\pm0.15^{a***}$	$9.9\pm0.45^{b***}$	$9.0\pm0.03^{c**}$
	Shigella sonnei	ICDDR'B	-	-	8.2±0.03
	Proteus mirabilis	ICDDR'B	$8.2\pm0.12^{a***}$	$9.5\pm0.09^{b***}$	-
	Escherichia coli	ICDDR'B	$9.0\pm0.11^{a***}$	$11.2\pm0.12^{b***}$	$22.0\pm0.03^{c***}$

*p<0.01, ***p<0.001*

good and compared well ($p < 0.001$) to that of the reference standard (tetracycline, 18.1, 22.0 and 27.0 mm). The oil showed lower activity against the other bacterial strains tested (ZOI, 7 - 13 mm). Only two bacterial species (*Klebsiella pneumoniae* and *Shigella sonnei*) showed resistance to the oil. On the other hand, *Proteus sp.* showed resistance to tetracycline but was sensitive to the oil.

The MIC of the essential oil against the bacterial strains tested ranged from 25 - 100 µl/ml (Table 3), with the lowest value (50 µl/ml) being for *B. polymyxa, B. megaterium* and *Proteus mirabilis*.

Table 3: Minimum inhibitory concentration (MIC) of the essential oil of *C. suffruticosa* leaf and tetracycline against some bacterial strains

Test organism	MIC (µl/ml)	
	Essential oil	**Tetracycline**
Gram-positive		
Bacillus subtilis	≥100	≥4
Staphylococcus aureus	≥75	≥16
Bacillus cereus	≥25	≥8
Bacillus polymyxa	≥50	≥8
Bacillus megaterium	≥50	≥8
Gram-negative		
Salmonella typhi	≥75	≥16
Shigella flexneri	≥100	≥16
Proteus mirabilis	≥50	≥8
Escheriae. Coli	≥75	≥4

Antifungal activity

The essential oil showed strong antifungal activity against all the test fungi (Table 4) and compared well with the standard antifungal, fluconazole (40 µg/disc). Specifically, the oil (16 µl/disc) achieved greater inhibition (40.8 %) against *Aspergillus ochraceus* than fluconazole (38.0 %). The highest inhibition by the oil was achieved against *Aspergillus niger* (62.2 %) at a concentration of 16 µl/disc. The data obtained were significant ($p < 0.001$) when compared with control.

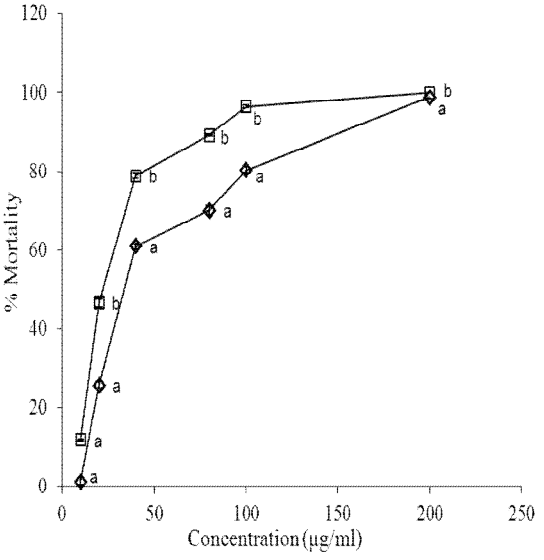

Figure 1: Lethality of the essential oil of *C. suffruticosa* leaf (▲) and reference standard, vincristine sulfate (■) against 24-h old brine shrimp (*A. Salina)*. Data are shown as mean ± SD of twenty shrimps for each concentration. Letters (a & b) shown in the plot indicate that the values are significantly different (Tukey multiple range *post hoc test*, $p < 0.05$) from each other (a compared to b); (****p<0.001).*

Cytotoxicity

Fig 1 shows that the LC_{50} value for the essential oil was 41.16 µg/ml, compared with the LC_{50} value of 0.76 µg/ml for the reference standard, vincristine sulfate. Regression analysis and probit analysis of the cytutoxicity data (Table 5) indicate that the essential oil possesses very strong cytotoxic effect to defend tumor cells, pests and other fungal cells.

DISCUSSION

Plant essential oils (volatile oils) and extracts have been used for a wide variety of purposes for thousands of years. Essential oils are generally variable mixtures of terpenoids, principally monoterpenes and sesquiterpenes. Although, diterpenes may also be present, a variety of low molecular

Table 4: *In-vitro* antifungal activity (mean ± SD) of the essential oil of *C. suffruticosa* leaf

Organism	Source ID	Inhibition (%)		
		Essential oil (10 µl/disc)	***Essential oil (16µl/disc)***	***Fluconazole (100µg/disc)***
A. ustus	DSM 63535	$26.85\pm0.07^{a***}$	$28.57\pm0.03^{b***}$	$40.16\pm0.16^{c***}$
A. niger	DSM 737	$59.21\pm0.13^{a***}$	$62.20\pm0.11^{b***}$	$64.00\pm0.16^{c***}$
A. ochraceus	DSM 824	$30.83\pm0.02^{a***}$	$40.80\pm0.02^{b***}$	$38.00\pm0.44^{c***}$

*Different superscript letters presented in the table are significantly different from each other (Tukey's multiple range post hoc test, **p<0.01, ***p<0.001).*

Table 5: Regression analysis of the cytotoxicity data for the essential oil of *C. suffruticosa* leaf and vincristine sulfate

Sample	95% confidence limit (µg/ml)	Regression equation	Chi square
Essential oil	31.66 - 52.33	y = 2.92 + 4.03x	1.58
Vincristine sulfate	0.57 - 0.82	y =3.16+2.98x	0.63

weight aliphatic hydrocarbons (linear, ramified, saturated and unsaturated), acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids are usually the constituents [8]. We identified in the present study 22 different types of chemically active secondary metabolites in *C. suffruticosa* essential oil.

The antimicrobial activity of the compounds of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [9]. The antimicrobial properties of essential oils and their constituents from a wide variety of plants have been assessed and reviewed to establish them as therapeutically important tools as well as their industrial applications, especially their potential in medical procedures and as ingredients in the cosmetic, food and drug manufacturing industries [10].

The efficacy of the essential oil against microorganisms usually depends on the nature of the major chemical constituents and their contribution to the oil. The mechanisms by which they inhibit/kill (or both) the microbes also vary to a large extent. The hydrophobic nature of essential oils make them to partition into the lipid bilayer of the cell membrane, affecting the respiratory chain and energy production of microorganisms, rendering it more permeable for the uptake of antibiotics and leading to leakage of vital cell contents. However, some compounds of essential oil, such as thymol and carvacrol, act as membrane permeabilizers, enhancing the intake of antibiotics [12]. Bacterial efflux pumps are energy-dependent (ATP) pumps that may be affected by these permeabilizers. In the present study, estragole (58.23 %), anethole (33.8 %) and linalool (3.38 %) were the major compounds found, constituting approximately 95 % of the oil. Coutinho *et al* [13] reported the antibacterial effect of estragole against *B. cereus, S. typhi* and *Staph. aureus* which showed some similarity to the findings of the present study. Anethole is said to have shown very promising activity against *E. coli, K. penumoniae, Staph. aureus, B. subtilis, B. megaterium and B. cereus*, and this is consistent with our results [13]. The activity of linalool against bacterial strains such as *Staph. aureus* and *B. cereus* [14] has also established. Some other minor constituents such as α-pinene are also effective against *Staph. aureus* and *E. coli* [15]. All these documented information are in agreement with our findings in the present work.

The essential oil exhibited very potent antibacterial activity against both Gram-positive and Gram-negative strains but the former was more susceptible than the latter. This is in agreement with previous studies which reported a similar finding [16]. This is also supported by the MIC results obtained in the present study. The weak antibacterial activity against Gram-negative bacteria has been attributed to the presence of an outer membrane containing hydrophilic polysaccharide chains that act as a barrier to essential oils which are hydrophobic [12]. *Klebsiella* did not show susceptibility to essential oil because it is an opportunistic pathogen most frequently associated with extended spectrum β-lactamase (ESBL) production [17]. Such organisms are usually resistant to most antibiotics and pose a serious threat in health care associated infections. Only essential oils rich in carvacrol and thymol, which were absent from *C. suffruticosa* oil, have gained importance for their antimicrobial activity against Gram-negative organisms. The fact that sensitivity to the oil varied with the bacterial strain suggests that the activity of the oil is due to a number of its components working synergistically [18].

The antifungal activity of *C. suffruticosa* essential oil was higher than that of antifungal drug, fluconazole. This may be related to the chemical structure of the constituents of the oil [16]. The magnitude of activity of the oil may be linked to the presence of an aromatic nucleus containing a polar functional group. However other factors such as the hydrophilic/hydrophobic balance are likely to be involved; for example, the phenolic-OH groups are very reactive and easily form hydrogen bonds with active sites of enzymes on the fungal cell wall [16]. Thus, the chemical characteristics of the major compounds in the oil, estragole and anethole (both of which are alcoholic in nature) are probably responsible for the potent antifungal activity of the oil. This is buttressed by the previous studies on other essential oils containing the same compounds [15].

It is reported that LC_{50} value higher than 1.0 mg/ml is considered as a cutoff point for a plant material to be non-toxic and that value less than 1000 µg/ml is detected as toxic in brine shrimp sensitivity test [19]. According to the observation, the obtained LC_{50} value for *C. suffruticosa* indicates the notable clinical importance of the essential oil for pharmaceutical formulation against tumor cells, pesticides etc. as the brine shrimp cytotoxicity assay is considered as a convenient probe for preliminary assessment of toxicity, detection of fungal toxins, pesticidal and anti-tumor effect [7, 20].

The rate of mortality of brine shrimp (*A. salina*) by essential oil is statistically significant ($p < 0.001$) in comparison to vincristine sulfate. The lethal concentration (LC_{50}) derived from regression and probit analysis [19] show that the LC_{50} of essential oil of *C. suffruticosa* leaves is 41.16 µg/ml, with the lower and upper limits being 31.66 and 52.33 µg/ml, respectively. Comparison of this result with the standard vincristine sulfate (0.76 µg/ml) indicates that the lethality of the essential oil is considerably lower ($p < 0.001$).

CONCLUSION

The essential oil of *C. suffruticosa* has demonstrates some potent antibacterial properties; however, Gram-positive bacteria are more susceptible to it than Gram-negative organisms. The oil also possesses stronger antifungal property than the synthetic conventional antifungal drug, fluconazole. The relatively low cytotoxicity of the oil suggests that it can be safely used in formulations containing the material.

ACKNOWLEDGEMENT

The authors wish to thank Industrial Microbiological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh, for making available the microbial strains and GC-MS analytical facility used.

REFERENCES

1. Hooker JD. Flora of British India, Vol. I, Reeve & Co. Ltd, England, 1875.
2. Rahman MA, Chakma JS, Islam S, Ahmed NU. Evaluation of antioxidant, antibacterial, antifungal and cytotoxic effects of Clausena suffruticosa ethanolic extract. J. App. Pharm. Sci. 2011; 1: 90-95.
3. Cornu A, Massot R. Compilation of Mass Spectral Data, Vols. 1 & 2, Heyden & Sons, London, 1975.
4. Pfleger K, Maurer HH, Weber A. Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites, 2nd ed., Wiley-VCH, Weinheim, (2000).
5. Akteruzzaman M, Sayeed A, Islam A, Khan GRMAM. Antimicrobial activity of chemical constituents of Pouzolzia indica Gaud. J. Bio Sic. 2001; 9: 27-30.
6. GroverRK, Moore JD. Toximetric studies of fungicides against brown rot organism-Sclerotinia fructicola and S.laxa. Phytopathol. 1962; 52: 876-880.
7. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med.1982; 45: 31-34.
8. Rūta M, Bakutis B, Baliukonienė V. Antimicrobial activity of two essential oils. Ann Agric Environ Med. 2011; 18: 139–144.
9. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. J. App. Bacteriol.1997; 82: 759-762.
10. Pélissier Y, Marion C, Casadebaig J. A chemical, bacteriological, toxicological and clinical study of the essential oil of Lippia multiflora mold (Verbenaceae). J. Essent. Oil Res. 1994; 6: 623-630.
11. Nicolson K, Evans G, O'Toole PW. Potentiation of methicillin activity against methicillin-resistant Staphylococcus aureus by diterpenes. FEMS Microbiol. Lett. 1999; 179: 233–239.
12. Mann CM, Cox SD, Markham JL. The outer membrane of Pseudomonas aeruginosa NCTC6749 contributes to its tolerance to the essential oil of Melaleuca alternifolia (tea tree oil). Lett. App. Microbiol. 2000; 30: 294–297.
13. Coutinho HDM, Matias EFF, Santos KKA, Tintino SR, Souza CES, Guedes GMM, Santos FAD, Costa JGM, Falcão-Silva VS, Siqueira-Júnior JP. Enhancement of the Norfloxacin Antibiotic Activity by Gaseous Contact with the Essential Oil of Croton zehntneri. J.Young Pharm.2010; 2: 362-364.
14. De M, De AK, Sen P, Banerjee AB. Antimicrobial properties of star anise (Illicium verum Hook f), Phytother. Res. 2002; 16: 94-95.
15. Gachkara L, Yadegaria D, Rezaeib MB, Taghizadehc M, Astanehc SA, Rasoolic I. Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils, Food Chem., 2007; 102: 898-904.
16. Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA. Antimicrobial activity of some Egyptian spice essential oils. J. Food Prot. 1989; 52:665–667.
17. N'guessan JD, Dinzedi MR, Guessennd N, Coulibaly A, Dosso M, Djman AJ, Guede-Guina F. Antibacterial activity of the aqueous extract of Thonningia sanguinea against Extended-Spectrum-ß-Lactamases (ESBL) producing Escherichia coli and Klebsiella pneumoniae strains. Trop. J. Pharm. Res. 2007; 6; 779-783.
18. Koutsoudaki C, Krsek M, Rodger A. Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of Pistacia lentiscus Var. chia. J. Agric. Food Chem. 2005; 53: 7681–7685.
19. Bliss CI. The method of probits. Sci. 1934; 79: 38-39.
20. Aseer M. Sujith S, Kiran SG, Joseph S, Chippu S. Cytotoxicity potential of Red alga, Laurencia brandenii collected from the Indian Coast. Global J. Pharmacol. 2009; 3: 90-94.