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Evaluation of Antibacterial Activity of Some Medicinal Plants Used in Sudanese Traditional Medicine for Treatment of Wound Infections

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Abstract: The ethanolic, petroleum ether, ethyl acetate, methanolic and water extracts of some medicinal plants (*Acacia nilotica* ssp. *nilotica* pods, *Lawsonia inermis* leaves, *Azadirachta indica* leaves, *Trigonella foenum-graecum* seeds and *Cordia sinensis* stem bark) were investigated for their antibacterial activity against six standard bacterial strains commonly associated with wound infections (*Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 1312) in vitro. Plant extracts at a concentration of 100 mg/ml were applied using the agar plate well- diffusion method. All the extracts obtained from *A. nilotica* ssp. *nilotica* pods were showed a range of activity against all tested bacterial strains. In particular, the ethyl acetate and methanol extracts showed the highest levels of activity. Among the tested extracts of *L. inermis*, the alcoholic extracts of the leaf showed the highest levels of activity. The leaf water extract of *A. indica* showed the highest levels of activity against *S. aureus*, *E. coli* and *P. aeruginosa*. The least antibacterially active plants were *Trigonella foenum-graecum* and *Cordia sinensis*. Most susceptible Gram-negative standard bacterium was *Klebsiella pneumoniae* and least susceptible Gram-negative bacterium was *Escherichia coli*. In Gram-positive bacteria, most susceptible bacterium was *S. aureus*. Compared to reference antibiotics, some plant extracts exhibited broader spectrum of antibacterial activity and were found to be clearly superior in case of extracts of *A. nilotica* ssp. *nilotica* and *L. inermis* against *K. pneumoniae*.

Key words: Antibacterial activity · Sudanese medicinal plants · wound infections

INTRODUCTION

Intensive use of antibiotics is often followed by the development of resistant strains. Because this drug resistance, the search for new antibiotics continues unabated. The interest in the study of medicinal plants as source of pharmacologically active compounds has increased worldwide. In Sudan, plants are the main medicinal source to treat infectious diseases. As part of continuous research in Botany Department, Faculty of Science and Technology, Omdurman Islamic University on biological properties of plants used in Sudanese traditional medicine, in this study we investigate in vitro antibacterial activity of different extracts from five plant species against six standard bacterial strains, which are common cause of wound infections.

MATERIALS AND METHODS

Plant Material: The pods of *Acacia nilotica* ssp. *nilotica*, the leaves of *Lawsonia inermis* and *Azadirachta indica* and the stem bark of *Cardia sinensis* were collected from EL-FITEHAB area, West White Nile, Omdurman Province. The plants were identified in the Botany Department, Faculty of Science and Technology, Omdurman Islamic University by one author, Dr. Hatil Elkamali and by comparison with herbarium of the Department. The plants were spread and dried in the shade for three weeks and then pulverized with a mechanical grinder.

Preparation of Plant Extracts: Different extracts were prepared by a modification of the method according to Robinson, [1]. The steps are graphically presented as a flow chart in Figure (1).

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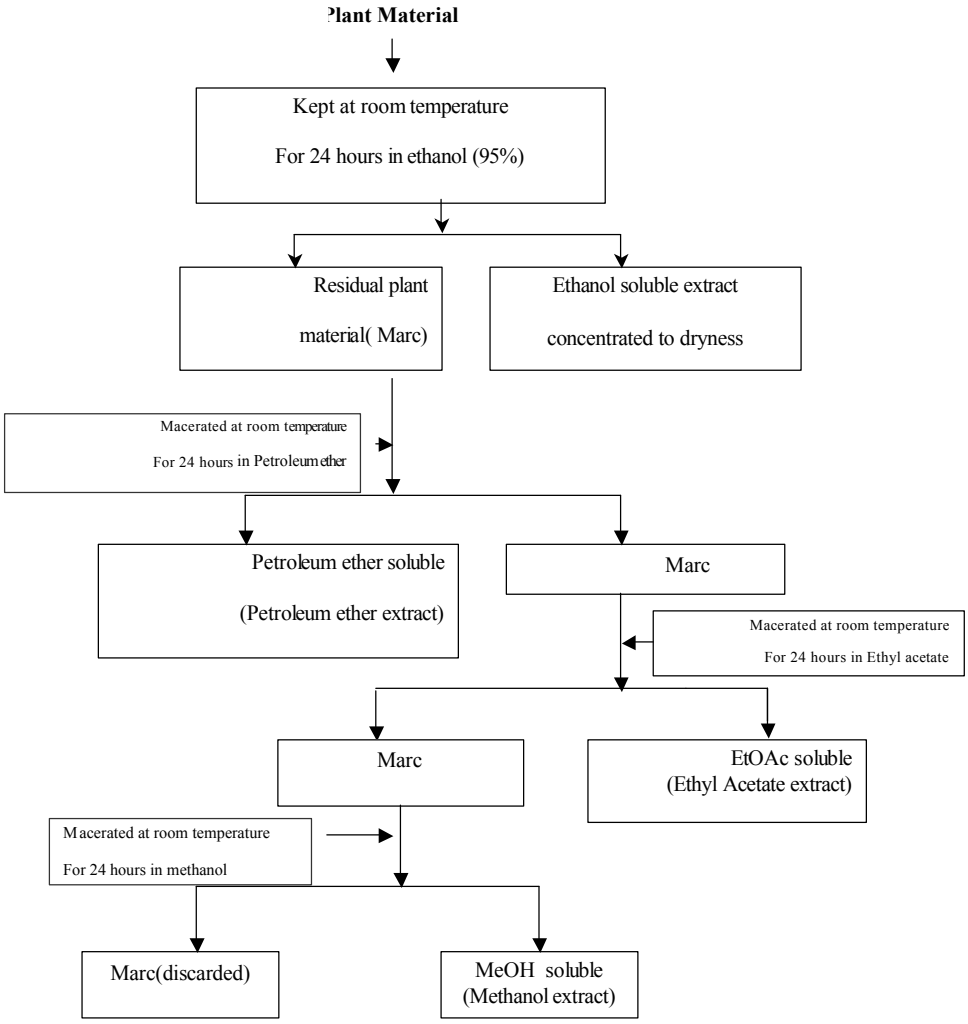

Fig. 1: Flow Chart for plant Extraction

Preparation of Aqueous Extracts: Air- dried plant material (100 g) was ground to a fine powder. It was poured with distilled water (1litre) and left for 24 hours at room temperature. The mother liquor was filtered. The filtrate, thus obtained was evaporated to complete dryness at room temperature. The residue thus obtained was aqueous plant extract. All extracts were stored dry in sterilized containers at room temperature until used for antibacterial testing. At the time of testing, the extracts were reconstituted to a concentration of 100mg/ml in Dimethyl Sulphoxide (DMSO).

Bacteriological Techniques: The bacteriological techniques followed were those described by Cheesbrough, [2]; Burdon and Williams, [3]; Brooks *et al*. [4]; Cruickshank *et al*. [5].

Preparation of Culture Media: Bacteria are generally grown in enriched culture media. Nutrient broth and nutrient agar were used to prepare enriched culture media and the methodology is given below.

Obtaining Pure Culture of Bacteria by Streak Plate Method: A loopful of bacterial cells was streaked across the agar-solidified surface for nutrient medium. The plates were then incubated under favourable conditions at 37°C. The key to this method is that, by streaking, a dilution gradient (a decreasing concentration of bacterial cells) was established across the surface of the plates, so that well isolated colony arises from a single bacterium and represents clone of a pure culture, which was then incubated at 37 °C for 24 hours to obtain pure colonies.

Tested Bacteria: Six standard bacterial strains were obtained from the American Type Collection Culture (ATCC) and National Collection Type Culture (NCTC). They were Gram positive (G+ve): *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236) and Gram negative (G-ve): *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 1312).

The bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4 °C. Subsequent cultivation and tests were done on nutrient agar medium.

Preparation of the Tested Bacteria: One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline and finally suspended in a small volume of normal saline to produce a suspension containing about 10^8-10^9 colony-forming units per ml. The suspension was stored in the refrigerator at 4°C until used.

The average number of viable organism per ml of the stock suspension was determined by means of the surface a viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline and 0. 02 ml volumes (one drop) of the appropriate dilutions were transferred by digital pipette (Finn pipette adjustable volume) onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0. 02 ml) was multiplied by 50 and the dilution factor expressed as the number of colony forming units per ml of suspension.

Each time a fresh stock suspension was prepared, all the above experimental condition were maintained constant so that suspensions with very close viable counts would be obtained.

Antibacterial Testing: The antibacterial activity was tested by well-agar diffusion method. 250 ml of sterilized nutrient agar was used for testing. The inoculum size of each test organism was adjusted to suspension of 10^6 cells. 2 ml ^{of}24 hours old culture of bacteria were added to ^{250} ml of melted cooled test agar and after through mixing, approximately 20 ml of this seeded agar were poured into 10 cm diameter presterilized petri dishes and allowed to solidify. Three wells (10 mm in diameter) were bored in the agar using a sterile cork borer and the agar discs were removed. 0. 1 ml aliquots of the reconstituted extract were placed into a well with a pipette and the plate was held for 2 hours at room temperature for diffusion of extract into agar. Subsequently, the plate was incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition was measured to the nearest mm. The minimum inhibitory concentration (MIC) was determined by a modification of the agar diffusion method according to Cruickshank *et al*. [5]. A two-fold serial dilution of each extract was prepared in DMSO (diluent) to achieve a decreasing range of extract concentrations from 100 mg/ml to approximately 3. 125mg/ml. A 0. 1 ml sample of each dilution was introduced into duplicate wells in a nutrient agar plate already seeded with bacterial cells as described above. Incubation was at 37°C for 24 hours. The lowest concentration of extract showing a zone of inhibition was taken as the MIC.

Gentamicin, Tetracycline and Ampicillin were used at concentrations ranging from 40 mg/ml to 5 mg/ml, as positive control and DMSO as a negative control.

RESULTS AND DISCUSSION

The antibacterial properties of the ethanol, petroleum ether, ethyl acetate, methanol and water extracts of some Sudanese medicinal plants (viz. *Acacia nilotica ssp nilotica* pods, *Lawsonia inermis leaves, Azadirachta indica leaves, Cordia sinensis* stem bark and *Trigonella foenum-greacum* seeds) at concentration 100 mg/ml were tested against six standard bacterial strains (*Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 1312). The results of diameter of the zones of inhibition are presented in Table 1.

Ethanol extracts of *Acacia nilotica ssp nilotica* pods and *Lawsonia inermis leaves* showed high antibacterial activity against all tested bacteria except *Lawsonia inermis* extract against *Pseudomonas aeruginosa* (ATCC 27853). Ethanol extract of *Azadirachta indica leaves* was found effective against one tested organism, *Bacillus subtilis* (NCTC 8236). Ethanol extract of *Cordia sinensis* stem bark showed promising result against *Klebsiella pneumoniae* (ATCC 1312) and, *Pseudomonas aeruginosa* (ATCC 27853).

All bacterial species were found to be resistant against *Trigonella foenum-greacum* seeds, except *Pseudomonas aeruginosa* (ATCC 27853).

Table 1: Antibacterial Activity of extracts from some medicnal plants against strains causing infections

Plant (Part)	Extract	S.a	B.s	E.c	P.v	P.a	K.p
Acacia nilotica ssp nilotica (Pods)	Ethanol Petroleum ether	27	27	26	25	23	24
	Ethyl acetate Methanol	20	22	23	21	21	24
	Aqueous	37	27	40	30	40	32
		36	29	37	32	35	32
		43	22	39	26	27	21
Lawsonia inermis (Leaves)	Ethanol Petroleum ether	29	20	24	22	-	17
	Ethyl acetate Methanol	-	-	-	-	-	-
	Aqueous	24	21	20	21	-	19
		28	20	26	22	-	19
		20	22	15	-	16	12
Azadirachta indica (Leaves)	Ethanol Petroleum ether	-	18	-	-	-	-
	Ethyl acetate Methanol	-	-	15	-	-	-
	Aqueous	18	14	-	-	-	-
		-	17	-	-	15	19
		22	17	19	-	22	-
Trigonela foenum-graecum (Seeds)	Ethanol Petroleum ether	-	-	-	13	15	-
	Ethyl acetate Methanol	-	-	-	-	-	-
	Aqueous	-	-	-	-	-	-
		-	-	-	-	-	-
		-	-	-	-	-	-
*Cordia sinensi*s (Stem bark)	Ethanol Petroleum ether	-	-	15	15	15	20
	Ethyl acetate Methanol	-	-	-	-	-	-
	Aqueous	-	-	-	-	-	-
		19	-	-	-	15	-
		-	-	-	-	-	-

S. a: *Staphylococcus aureus* (ATCC 25923), B. s: *Bacillus subtilis* (NCTC 8236)
E. c: *Escherichia coli* (ATCC 25922), P. a: *Pseudomonas aeruginosa* (ATCC 27853)
P. v: *Proteus vulgaris* (ATCC 6380), K. p: *Klebsiella pneumoniae* (ATCC 1312)
Values are the mean of three replicates of inhibition zone (mm);
Tested concentration of extracts: 100 mg/ml (0. 1 ml/well)
DMSO did not show any inhibitory activity
Mean Inhibition Zone Diameter (MIZD):
sensitive: >18 mm; intermediate 14-18mm and resistant <14 mm.

Table 2: Antibacterial activity of antibiotics against different standard group of bacteria

		Test organisms					
Antibiotics	Concentration (microgram/ml)	S.a	B.s	E.c	P.v	P.a	K p
Gentamicin	40	16	19	22	18	15	20
	20	12	15	18	15	13	18
	10	-	12	15	-	-	13
	5	-	-	12	-	-	-
Tetracycline	40	15	22	16	-	16	20
	20	12	20	15	-	13	18
	10	-	18	14	-	-	16
	5	-	16	12	-	-	15
Ampicillin	40	-	-	15	-	18	26
	20	-	-	-	-	15	23
	10	-	-	-	-	-	20
	5	-	-	-	-	-	19

Table 3: Minimum Inhibitory Concentration (MIC) of Extractive from *Acacia nilotica ssp nilotica* and *Lawsonia inermis*

		Test organisms					
Plant species	Extract	S.a	B.s	E.c	P.v	P.a	K p
Acacia nilotica ssp nilotica	Ethanol	6.25	‹3.125	12.5	25	‹3.125	‹3.125
	Petroleum ether	50	25	50	50	50	12.5
	Ethyl acetate	25	‹3.125	25	25	25	6.25
	Methanol	‹3.125	6.25	12.5	12.5	6.25	‹3.125
Lawsonia inermis	Ethanol	25	25	12.5	12.5	6.25	‹3.125
	Petroleum ether	-	-	-	-	-	-
	Ethyl acetate	25	25	6.25	25	6.25	‹3.125
	Methanol	50	50	50	50	50	12.5

Petroleum ether extract of *Azadirachta indica* was found moderately active against *Escherichia coli* (ATCC 25922).

Ethyl acetate extract of *Acacia nilotica ssp. nilotica* pods, *Lawsonia inermis leaves* showed high antibacterial activity against all tested bacteria (except *Lawsonia inermis* extract against *Pseudomonas aeruginosa* (ATCC 27853). Ethyl acetate extract of *Azadirachta indica leaves* showed antibacterial activity against Gram-positive bacterial strains.

Most of the bacterial species showed a high degree of sensitivity to the methanolic extracts of *Acacia nilotica ssp nilotica* pods (1Z = range between 21-37 mm) and *Lawsonia inermis leaves* (1Z = range between 19-28 mm) except against *Pseudomonas aeruginosa* (ATCC 27853).

Extract of *Azadirachta indica leaves* was found effective against *Klebsiella pneumoniae* (ATCC 1312), *Bacillus subtilis* (NCTC 8236) and *Pseudomonas aeruginosa* (ATCC 27853).

Stem bark extract of *Cordia sinensis* showed antibacterial activity against *Staphylococcus aureus* (ATCC 25923) (1Z = 19 mm) and *Proteus vulgaris* (ATCC 6380) (1Z = 15 mm)

Different extracts of *Trigonella foenum-greacum* seeds did not show any activity against all tested Gram-positive and Gram-negative bacteria.

Aqueous extract of *Acacia nilotica ssp nilotica* pods was highly active against all tested Gram-positive and Gram-negative bacteria (1Z = range between 21-43 mm).

Leaf extract of *Lawsonia inermis* showed antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) (1Z = range between 15-22 mm), but no growth Inhibition was observed against *Proteus vulgaris* (ATCC 6380) and *Klebsiella pneumoniae* (ATCC 1312).

Aqueous extract of *Azadirachta indica* leaves was found effective against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (NCTC 8236), but no activity was observed against *Proteus vulgaris* (ATCC 6380) and *Klebsiella pneumoniae* (ATCC 1312).

All bacterial species were found to be resistant against extract of *Cordia sinensis* stem bark and *Trigonella foenum-greacum* seeds.

The antibacterial activity related to known antibiotics was calculated (Table 2). Gentamicin was found effective against all tested bacteria at concentration 40 ìg/ml. Tetracycline showed good results against *Bacillus subtilis* (NCTC 8236) and *Klebsiella pneumoniae* (ATCC 1312) at concentration 40 and 20 ìg/ml.

Staphylococcus aureus (ATCC 25923), *Bacillus subtilis* (NCTC 8236) and *Proteus vulgaris* (ATCC 6380) was found to be Amp – resistant.

Based on the initial antibacterial screening test, *Acacia nilotica ssp. nilotica* and *Lawsonia inermis* were selected for further studies for the determination of MIC, because they were found to be most active against bacterial strains. The MICs of the extracts are shown in Table 3.

As can be seen from the results, *Acacia nilotica ssp nilotica* is the most active species against bacteria. All species of plants included in the present study were also found to be active on at least one of the selected microbial strains.

The antibacterial activity profile of *A. nilotica ssp nilotica* and *Lawsonia inermis* extract against the tested strains indicated that *Escherichia coli* and *Klebsiella pneumoniae* were the most susceptible bacterium of all the bacterial test strains.

In fact, Gram- negative bacteria are frequently reported to have developed mulit-drug resistance to many of the antibiotics currently available in the market of which *Escherichia coli* is the most prominent [6, 7]. Therefore, it is not surprising to learn that *E. coli* is the least responding bacterial strain to the tested plant extracts. However, some extractives of plants are still of special interest for further investigation in this regard as in the case of methanol and ethanol extracts of *Lawsonia inermis*, water extract of *Azadirachta indica* and ethyl acetate, ethanol, methanol and water extracts of *Acacia nilotica ssp. nilotica* it showed exceptionally stronger activity against *E. coli* than other plant extracts yet having moderate/ poor activity on Gram-positive bacteria, a trend not observed for other species of plants. The antibacterial activity *Acacia nilotica ssp. nilotica*, *Lawsonia inermis* and *Azadirachta indica* extracts was more pronounced on the Gram-positive bacteria (*Staphylococcus aureus*) than the Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be ascribed to the differences in morphological constitutions between

these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell wall of Gram-negative organisms which are more complex than the Gram-positive ones act as a diffusional barrier and making them less susceptible to the antimicrobial agents than are Gram-positive [8, 9]. In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against Gram-negative organisms as well.

The MIC values indicate that the methanolic extract of *Acacia nilotica ssp. nilotica* is more potent against *Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* bacteria and similarly, ethanolic and ethyl acetate extracts of *L. inermis* found to be more active against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The results were in agreement with the initial antimicrobial screening test results. *Klebsiella pneumoniae* was more sensitive to the antimicrobial agents (extracts) from among the Gram-negative bacteria being inhibited at ‹3. 125 mg/ml by both *Acacia nilotica ssp. nilotica* and *Lawsonia inermis* crude extracts.

The appraisal of obtained results shows that all extracts of *Acacia nilotica* ssp. *nilotica* pods and *Lawsoina inermis* leaves (except petroleum ether) were highly effective against all tested bacteria. Further work is need to identify compound/compounds responsible for antibacterial activity. Further studies on other bacteria and some pathogenic fungi frequently isolated from burn wound specimens such as *Enterobacter cloacae*, *Enterococcus faecalis*, *Aspergillus spp*. and *Candida albicans* would be interesting.

REFERENCES

1. Robinson, T., 1963. The organic constituents of higher plants: their chemistry and interrelationships. USA. Burgess Publishing Company, pp: 306.
2. Cheesbrough, M., 1984. Culture Media. In: Medical Laboratory Manual for Tropical Countries. Tropical Health Technology and Butterworth-Heineman, Cambridge. Vol. III, 60(69): 407-428.
3. Burdon, K.L. and R.P. Williams, 1968. Microscopes and microscopic method. In: Microbiology. 6th ed. The Macmillan Company. London, pp: 74-76.
4. Brooks, G.F., J.S. Butel and S.A. Morse, 2001. Antimicrobial chemotherapy. In: Medical Microbiology. 22nd ed. Appleton and Large. USA. 170: 222-223.
5. Cruikshank, R., 1975. Medical Microbiology: AGuide to Diagnosis and Control of Infection. 1st ed. Edinburgh and London: E and S Livingston Ltd. pp: 888.
6. Alonso, R., A. Femandez-Aranguiz, K. Colorn, A. Herreras and R. Cistema, 2000. Profile of Bacterial Isolates and antimicrobial susceptibility: Multi center study using a one-day cut-off. Revista Espnola de quimioterapia, 13: 384-393.
7. Sader, H.S., R.N. Jones and J.B. Sibu, 2002. Skin and soft tissue infections in Latin American Medical Centers: Four year Assessment of the pathogenic frequency and antimicrobial susceptibility patterns. Diagnostic Microbiology of Infectious Diseases, 44: 281-288.
8. Nostro A., M.P.D. Germano, V. Angelo, A. Mavino, M.A. Cannatelli, 2000. Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. Letters in Appl. Microbiol., 30: 379-384.
9. Hodges, N., 2002. Pharmaceutical Applications of Microbiological Techniques. In: Aulton, M. E., (Ed.). Pharmaceutics: The Sciences of Dosage from Design. 2nd ed. Harcourt Publishers Ltd. London, pp: 606.