

## **Pytochemical screening and evaluation antimicrobial activity of the methanol extract of *Ficus carica***

Hakima Belattar<sup>§</sup>  
Sara Himour  
Abdelouahab Yahia

Department of Natural and Life Sciences-Laboratory of Natural Sciences and Materials-University Center Abdelhafid Boussouf Mila. BP N° 26, Mila. ZC. 43000. Algeria.

<sup>§</sup>Corresponding author: h.belattar@centre-univ-mila.dz.

### **Abstract**

Fig tree (*Ficus carica* Linn.) was appreciated as food and for its medicinal properties, it grows in Mediterranean region, and it is admirably adapted to the conditions of Algeria. The use of natural resources to treat and cure diseases is an old and still widespread method. The objective of this work was to evaluate the antibacterial activity that exists through methanolic extracts of fig leaves grown in the Algerian environment. Antibacterial assay was carried out via disc diffusion method to measure the diameter of the zone of inhibition on the Müller-Hinton agar plate against four selected bacteria strains *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsiella pneumonia* (Gram negative), in addition to the detection of some active compounds was carried out by phychemical screening. The result obtained showed that *F. carica* extracts revealed the presence of flavonoids, saponins, tannin, alkaloids. The presence of secondary metabolites made in these extracts is the cause of the observed antimicrobial potential. Consequently, all extracts exhibited the bactericidal effect towards the bacteria tested, while the crude extract of methanol was active against Gram positive bacteria more than Gram negative bacteria. In this study, the potential for development of alternative antibiotics derived from the methanolic extract of fig leaves was highlighted.

**Key words:** *Ficus carica* Linn., antibacterial activity, methanol.

Reception date: November 2020

Acceptance date: February 2021

## Introduction

Plants have been consistently a source of natural products for the treatment of various diseases (Newman and Cragg, 2005). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from them (Arunkumar and Muthuselvam, 2009). In recent years, plant secondary metabolites (phytochemicals) that had previously unknown pharmacological activities have been studied in detail as a source of drugs (Krishnaraju, 2005). Therefore, phytochemicals with sufficient antibacterial activity should be used to treat bacterial infections (Balandrin, 1985).

Infectious diseases remain a critical problem for human health, as antibiotics and vaccination programs are used (Adeshina *et al.*, 2010). Many bacteria presently present a huge problem as multidrug resistance may have developed against the antimicrobial agents used against the infection, making the discovery of new antimicrobial agents an undesirable effect (Chanda and Kaneria, 2011).

Increasing resistance to antibiotics and the failure of many chemotherapeutic agents have led to the study of medicinal plants for their antimicrobial effect (Colombo and Bosisio, 1996). Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (Cseke *et al.*, 2006).

Unlike synthetic drugs, the antimicrobial property that comes from traditional plants has no side effects and has a high potential for curing infectious diseases (Blesson *et al.*, 2015). Herbal medicines have their own benefits for human health which require further research and investigation. The fig, *Ficus carica* L., belongs to family Moraceae, all parts of this plant such as bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important (Salem *et al.*, 2013).

Adeshina *et al.* (2010) found that the high antimicrobial activity of *Ficus* spp., is due to the frequency of flavonoid compounds in the leaves. The flavonoid and phenol content of fig leaves shows antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee and Jeong, 2010). In addition, this medicinal plant also contains many bioactive compounds such as vitamins and flavonoids (Joseph and Raj, 2011).

The main objective of the present study was to determine the antimicrobial potential of different concentrations of leaves crude extracts of *F. carica* grown in Algeria environments against selected food borne pathogenic bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*k. pneumonia*) and *Escherichia coli* (*E. coli*).

## Materials and methods

### Sampling site and study area

The sampling site was Emdjez-Edchiche town 32 km north of Skikda (North-East Algeria). The varieties were collected from fig collection in the Technical Institute of Fruit Trees (ITAF) and confirmed by Belattar *et al.* (2017). Evaluation of the antimicrobial activities of the fig

extracts and phytochemical constituency was carried out in Natural Sciences and Materials Laboratory, Department of Natural and Life Sciences, University Center Abdelhafid Boussouf Mila.

### **Preparation of the methanol extract**

The dried (60 °C, 48 h) and finely ground samples of leaves (6 g) were separately extracted with 35 ml of 100% methanol for 12 h at room temperature with shaking. The plant materials were extracted twice in the same conditions after filtration. The methanol extracts obtained from each sample were collected, filtered, dried under vacuum and then re-dissolved in methanol and stored under refrigeration for further analysis (Changwei, 2008) with minor modifications.

### **Phytochemical screening**

The extracts were subjected to phytochemical tests for leaves secondary metabolites, tannins, saponins, steroid, alkaloids, flavonoid, triterpen in accordance with Trease *et al.* (1989); Harborne (1989).

### **Antibacterial activity**

The antibacterial activity of methanolic leaves extract against various Gram positive and Gram negative bacteria were observed. Reference strains were: *Staphylococcus aureus* (ATCC 25293), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603), were obtained from the laboratories of University Center Abdelhafid Boussouf Mila, Algeria.

### **Disc diffusion method**

Disc diffusion method was done based on (Murray *et al.*, 1995). The strains of microorganism obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37 °C or 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar, poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs.

Sterile discs of six millimeter with had been impregnated with 20 µl of test extract at different concentrations: 25 mg ml<sup>-1</sup>, 50 mg ml<sup>-1</sup> and 100 mg ml<sup>-1</sup> and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 °C. Negative controls were prepared using Dimethylsulfoxide (DMSO). Gentamicin was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibition surrounding the wells in millimeter (mm) against the test organisms.

## Result and discussion

### Phytochemical screening

The result obtained from the phytochemical screening of methanolic extracts of selected Algerian figs is given in the (Table 1), showed the present of flavonoids, saponins, tannin, alkaloids in varying concentration, while the absence of steroids and triterpenoids. These phytochemical compounds are known to be bioactive compounds and all play a role for antibacterial activity of *Ficus carica* extracts.

**Table 1. Phytochemical constituents of methanolic extract of selected Algerian figs.**

Ecotypes	Flavonoids	Saponins	Tannins	Alcaloids	Steroids	Triterpenoids
Albo	+	+++	++	+	-	+
Celeste	+	++++	+++	+	-	+
Cavaliere	+	+++	+++	++	-	+
'Boule d'or'	++	+++	+++	+	-	+
'Blanquette'	+++	+++	++	++	-	+++
Blak dourou	+	+++	+++	+	-	+++
Bifer de tala amara	++	++++	++	++	-	+
Bakor blanc	+	+++	++	+++	-	++
Avoacou	++	++	+++	++	-	+
Alekak	+	+	+++	-	-	+
Abiarous	+	+++	+++	+	-	+++
Fraga	+	+	++	+	-	+

-= absent; += faintly present; += moderately present; +++= highly present.

### Antibacterial activity

The result of the antibacterial activity of leaves methanolic extracts of *F. carica* are indicated in the Table 2. Present diameters of inhibition zones exerted towards tested microorganisms at different concentrations (25, 50, 100  $\mu\text{g ml}^{-1}$ ). It showed that the extrat of 'Blanquette' cultivar leaves exhibited strong activities against the Gram positive bacteria (*P. aeruginosa* with 9, 25 mm in the diameter as inhibition zone) at the concentration of 100  $\text{mg ml}^{-1}$ , followed by 8.75 mm and 8mm at the concentration of 50  $\text{mg ml}^{-1}$  and 25  $\text{mg ml}^{-1}$  respectively.

In addition, it was highly affected against *S. aureus* which recorded 8.25 mm with 50  $\text{mg ml}^{-1}$ , followed by 7.75 mm, 7.12 mm at the concentration of 25  $\text{mg ml}^{-1}$  and 100  $\text{mg ml}^{-1}$  respectively. *E. coli* appeared to be less sensitive to the extracts, the inhibition zone was 6.5 mm, at the concentration 25  $\text{mg ml}^{-1}$  and resistant antibacterial activity was showed by 6 mm.

**Table 2. Antimicrobial activity of methanol extract from *Ficus carica* against the bacterial strains tested.**

Microorganisms	Inhibition zone diameters (mm)						DMSO	Gentamicin
	<i>Blanquette</i> (mg ml <sup>-1</sup> )			<i>Boule d'or</i> (mg ml <sup>-1</sup> )				
	100	50	25	100	50	25		
<i>E. coli</i>	6	6	6.5	6	6	6	T-	T+
<i>P. aeruginosa</i>	9.25	8.75	8	6.12	8	6.5	6	32
<i>K. pneumonia</i>	6	6	6	6	6	6	6	16.3
<i>S. aureus</i>	7.12	8.25	7.75	7	6	6	6	38.75

On the other hand, 'Boule d'or cultivar leaves showed the highest zone of inhibition against *in vitro* growth of *P. aeruginosa* with 8 mm at the concentration 50 mg ml<sup>-1</sup>, followed by 6.5 mm and 6.12 mm at the concentration 25 mg ml<sup>-1</sup>, 100 mg ml<sup>-1</sup> respectively. Furthermore, it was highly affected by *S. aureus* with 7 mm at the concentration 100 mg ml<sup>-1</sup>. Moreover *E. coli* appeared to be resistant to the extracts, the inhibition zone was 6 mm. As compared with control treatment the antibiotic Gentamicin, all the previous inhibition zone values were lower than the positive control (49 mm with *E. coli*, 32 mm with *P. aeruginosa* and 38, 75 mm with *S. aureus*)

The results of this study show that the bacterial strains studied have sensitivity towards the fig leaves extracts studied, which is manifested by the appearance of a zone of inhibition (Table 3). The highest inhibitory effect is obtained with the extract of the variety Abiarous vis-à-vis *k. pneumonia*, and the variety *Fraga* and *Cavaliere* with *P. aeruginosa* and *S. aureus* respectively.

**Table 3. Antibacterial activity of the methanol extract (100 mg ml<sup>-1</sup>) of *Ficus carica* vis-à-vis the bacterial strains tested.**

Cultivars	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>	<i>S. aureus</i>
<i>Albo</i>	6	6	12.75	6
<i>Celeste</i>	6	6	12.25	7
<i>Cavaliere</i>	6	6	10.25	8.25
<i>Blak dourou</i>	6	6	8.75	6
<i>Bifer de talaamara</i>	6	7	7.25	7
<i>Bakor blanc</i>	6	7	7	6
<i>Avoacou</i>	6	6	13.75	7.5
<i>Alekak</i>	6	7	9.25	6
Abiarous	6	6	15.75	6
<i>Fraga</i>	6	8.25	7.25	7.5

The zones of inhibition measured for the strain *k. pneumonia* range from 7 to 15.75 mm. The highest value is recorded by the extracts of the *Abiarous* variety with a diameter of 15.75 mm, followed by the varieties *Avouacou*, *Celeste* and *Cavaliere* with diameters of approximately 13.75, 12.25 and 10.25 mm, respectively. The white *Bakor* variety has the weakest zone of inhibition with a diameter of the order of 7 mm.

For the *Staphylococcus aureus* strain, better inhibition is observed by the extracts of the *Cavaliere* varieties with a diameter of the order of 8.25. While the varieties *Avouacou* and *Fraga* gave the same zone of the order of 7.5 mm. The zones of inhibition measured for the *P. aerginosa* strain vary from 6 to 8.25 mm. The highest value is recorded by extracts of the *Fraga* variety with a diameter of 8.25 mm. Followed by *Bifer de talaamara* and *Alekak* varieties with a 7 mm area. In addition, *E. coli* appeared to be resistant to the extracts, the zone of inhibition was 6 mm.

## Discussion

Phytochemical screening of methanolic extracts of *F. carica* leaves of different Algerian varieties revealed their richness in flavonoids and polyphenols with significant variability between the varieties tested (Mahmoudi *et al.*, 2016).

As several reports have shown that some flavonoid compounds demonstrate antibacterial activity against oral bacteria (Jeong *et al.*, 2009; Rashid *et al.*, 2014), they generally considered that flavonoids in *F. carica* may be related to antibacterial effects. This is explained by Salem *et al.* (2013), who stated that the leaves of *Ficus carica* have higher flavonoid content, so they contribute more to the antibacterial effects than to other parts of the plant. In addition, flavonoids and tannins are currently of great scientific interest as they are considered potent antibacterials, antioxidants, antifungals and antivirals (Chen *et al.*, 2008).

The results show that the inhibition zone produced by the positive controls (Gentamicin) was greater than that produced by all hydromethanolic extracts. This can be attributed to the fact that raw plant extracts contain lower concentrations of bioactive compounds (Chew *et al.*, 2012).

There are several studies on the antibacterial potential of crude extracts of *F. carica*. Jung reported that the crude methanol extract from leaves of *F. carica* had high potential against *E. coli*, but low potential against *S. aureus* (Jung, 2007). Another good study demonstrated that the variation in antibacterial potential was due to some flavonoid compounds in leaves of *F. carica* (Jeong *et al.*, 2005). Ahmed and Khan (2013) reported that the antibacterial potential of methanolic leaves extracted from *F. carica* against five bacterial strains *Bacillus cereus*, *Enterobacter aerogenes*, *Klebsial pneumoniae*, *Bacillus subtilis*, *Staphylococcus epidermidis* at different concentrations was found in descending order following *Staphylococcus epidermidis* > *Klebsielle pneumoniae* > *Bacillus subtilis* > *Bacillus cereus* > *Enterobacter aerogenes*.

The antibacterial potential by disc diffusion assay of agar revealed that the crude methanol extract of *F. carica* presented a potential against non-pathogenic bacteria as well as pathogens. The authors also mentioned that the significant effect on growth inhibition of Gram positive and Gram negative bacteria (Ahmed and Khan, 2013).

Mi-Ran *et al.* (2009), found the same results with methanolic extracts of fig leaves (*F. carica*) against oral bacteria, they indicated that the latex and extracts were raised against Gram positive bacteria compared to Gram negative bacteria (Mi-Ran *et al.*, 2009).

This antibacterial effect was directed against both pathogenic and non-pathogenic bacteria. The significant effect on growth inhibition of Gram positive and Gram negative bacteria has also been noted (Ahmad and Khan, 2013). Rashid *et al.* (2014) found the same results with the ethanolic extract of *F. carica* leaves (Rashid *et al.*, 2014). Al Askari *et al.* (2012) also showed that the ethanolic extract had a strong antimicrobial activity, with a maximal zone of inhibition noted against *Staphylococcus epidermidis* (21 mm) with MIC 25 µg ml<sup>-1</sup>. Ethanolic extracts also showed strong activity against fungal strains (Rashid *et al.*, 2014).

*Ficus carica* leaves show high antimicrobial activity of the methanolic extract against oral bacteria. The combined effects of MeOH extract with ampicillin or gentamicin are synergistic against oral bacteria (Jeong *et al.*, 2009). This synergistic effect was also observed when the methanolic extract of *F. carica* was combined with oxacillin or ampicillin against methicillin-resistant *Staphylococcus aureus* (Lee and Cha, 2010). Al-Yousuf (2012) visualized the antibacterial activity of the methanolic extract of *F. carica* was more effective against *B. megaterium* in Gram positive strains and *E. coli* in Gram negative strains.

Lee and Cha (2010) also observed the effect of the methanolic extract of *F. carica* with respect to *S. aureus*. This bacterium has a high sensitivity to antibiotics and substances with such potential, used as a bioindicator to analyze this property. According to Lazrag Aref *et al.* (2010), the antimicrobial activity of *F. carica* latex extracts varies with the extraction solvent; it is elevated with chloroform and ethyl acetate which are better antimicrobial extraction solvents.

The mechanism of the antimicrobial effects of polyphenols is very complex. Among the hypotheses advanced, we can mention the inhibition of extracellular microbial enzymes, the blocking of the substrate necessary for microbial growth or the chelation of metals such as iron and the inhibition of microbial metabolism (Milane, 2004).

## Conclusions

In conclusion, the results obtained in this study provide a rationale for the use of *F. carica* in traditional medicine as treatment of bacterial infections and the possibility of developing alternative antibiotics derived from the metabolic extract of fig leaves against pathogenic bacteria. For that, suggest further investigations of these extracts and their components on *in vivo* antioxidant activity prior to clinical use.

## Cited literature

- Adeshina, G.; Onaolapo, J.; Ehinmidu, J. and Odama, L. 2010. Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria. *Med. Plant. Res.* 4(8):649-658.
- Ahmad, J. and Khan, I. 2013. Evaluation of antioxidant and antimicrobial activity of *Ficus carica* leaves: an in vitro approach. *J. Plant. Pathol. Microbiol.* 4(1):157-160.
- Al-Askari, G.; Kahouadji, A.; Khedid, K.; Ouaffak, L.; Mousaddak, M.; Charof, R. and Mennane, Z. 2012. In vitro antimicrobial activity of aqueous and ethanolic extracts of leaves of *Ficus carica* collected from five different regions of Morocco. *J. Mater. Environ. Sci.* 4(1):33-38.

- Al-Yousuf, H. H. H. 2012. Antibacterial activity of *Ficus carica* L. extract against six bacterial strains. *Int. J. Drug. Dev. Res.* 4(4):307-310.
- Arunkumar, S. and Muthuselvam, M. 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World J. Agric. Sci.* 5(5):572-576.
- Balandrin, M. F.; Kjocke, A. J.; Wurtele, E. 1985. Natural plant chemicals: sources of industrial and mechanical materials. *Science.* 228(4704):1154-1160.
- Belattar, H.; Yahia, A.; Nemli, S.; Ates, D.; Erdogmus, S.; Ertan, B.; Himour, S.; Hepaksoy, S. and Tanyolac, M. B. 2017. Determination of the population structure of fig genotypes from Algeria and Turkey using inter primer binding site-retrotransposon and simple sequence repeat markers. *Agri. Sci. (AS).* 8(12):1337-1357.
- Blesson, J.; Saji, C. V.; Nivya, R. M. and Kumar, R. 2015. Synergistic antibacterial activity of natural plant extracts and antibiotics against methicillin resistant *Staphylococcus aureus* MRSA. *J. Mater. Environ. Sci.* 4(3):741-763.
- Chanda, S. and Kaneria, M. 2011. Indian nutraceutical plant leaves as a potential source of natural antimicrobial agents, in science against microbial pathogens: communicating current research and technological advances. Mendez-Vilas A. 2<sup>nd</sup>. (Ed). 1251-1259 p.
- Changwei, A. O.; Anpin, Li.; Abdelnaser, A. E.; Tran, D. X. and Shinkichi, T. 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil extract. *Food Control.* 19(10):940-948.
- Chen, H. Q.; Jin, Z. Y.; Wang, X. J.; Xu, X. M.; Deng, L. and Zhao, J. W. 2008. Luteolin protects dopaminergic neurons from inflammation-induced injury through inhibition of microglial activation. *Neurosci. Lett.* 448(2):175-179.
- Chew, A. L.; Jeyanthi, J. A. J. and Sasidharan, S. 2012. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pac. J. Trop. Biomed.* 2(3):176-180.
- Colombo, M. L.; Bosisio, E. 1996. Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). *Pharmacol. Res.* 33(2):127-134.
- Cseke, L. J.; Kirakosyan, A.; Kaufman, P. B.; Warber, S. L.; Duke, J. A. and Brielmann, H. L. 2006. Natural product from plants. 2<sup>nd</sup>. (Ed). CRC Taylor & Francis Group. ISBN: 0-8493-2976-0.
- Harborne, J. B. 1989). Phytochemical methods a guide to modern techniques of plant analysis. *Int. J. Pharm. Sci. Res.* 2(7):1669-1678.
- Jeong, M. R.; Cha, J. D. and Lee, Y. E. 2005. Antibacterial activity of Korean fig (*Ficus carica* L.) against food poisoning bacteria. *Korean. J. Food. Cookery. Sci.* 21(1):84-93.
- Jeong, M. R.; Kim, H. Y. and Cha, J. D. 2009. Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. *J. Bacteriol. Virol.* 39(2):97-102.
- Joseph, B. and Raj, S. J. 2011. Pharmacognostic and phytochemical properties of *Ficus carica* linn-an overview. 3(1):8-12.
- Jung, E. K. 2007. Antibacterial activity of extract and fractions from *Dryaria fortune* against oral bacteria. *J. Bacteriol. Virol.* 37(2):61-68.
- Krishnaraju, A. V.; Rao T. V. N. and Sundararaju, D. 2005. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int. J. Appl. Sci. Eng.* 3(2):125-134.
- Lazreg, Aref, H.; Bel-Hadj; Salah, K.; Chaumont, J. P.; Fekih, A.W.; Aouni, M. and Said, K. 2010. *In vitro* antimicrobial activity of four *Ficus carica* latex Fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). *Pak. J. Pharm. Sci.* 23(1):53-58.



- Lee, Y. and Jeong, D. 2010. Synergistic antibacterial activity of fig (*Ficus carica*) leaves extract against clinical isolates of methicillin-resistant *Staphylococcus aureus*. Korean J. Microbiol. Biotechnol. 38(4):405-413.
- Mahmoudi, S.; Khali, M. A. and Mahmoudi, N. 2016. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). Nature & Technologie. b- Sci. Agro. Biol. 5(2):35-40.
- Milane, H. 2004. La quercétine et ses dérivés molécules à caractère prooxydant ou capteurs de radicaux libres; études et applications thérapeutiques. Thèse de doctorat en Sciences Domaine: Pharmacochimie. Université Louis Pasteur Strasbourg.
- Mi-Ran, J.; Hye-Young, K. and Jeong-Dan, C. 2009. Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. J. Bacteriol. Virol. 39(2):97-102.
- Murray, P. R.; Baron, E. J.; Pfaller, M. A.; Tenover, F. C. and Tenover, R. H. 1995. Manual of clinical Microbiology. 6<sup>nd</sup> (Ed.). Washington. DC, ASM. 300 p.
- Newman, D. J.; Cragg, G. M. 2005. *In: drug discovery, therapeutics, and preventive medicine.* Zhang, L.; Fleming, A.; Demain, A. L. Editors. Humana Press; Totowa, NJ, USA. 74 p.
- Rashid, K. I.; Mohammd-Mahdi, N.; Alwan, M. A. and Burhan-Khalid, L. 2014. Antimicrobial activity of fig (*Ficus carica* Linn.) leaf extract as compared with latex extract against selected bacteria and fungi. J. Babylon University. Pur. Appl. Sci. 22(5):1620-1626.
- Salem, M. Z.; Salem, A. Z.; Camacho, L. M. and Ali, H. M. 2013. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: an over view. Afr. Microbiol. Res. 7(33):4207-4219.
- Trease, G. E. and Evans, W. C. 1989. Textbook of pharmacognosy. 12<sup>nd</sup> (Ed). Balliere, Tinadl: London. 343-388 pp.