

Phytochemical and Antimicrobial Activity of *Panicum turgidum* (Thummam) as a Grazing Herb against Some Animal Pathogens

Rehab Mohamed Atta El-Desoukey*

Microbiology and Immunology Department, National Research Center, Giza, Egypt / Shaqraa University, KSA

*Corresponding Author: Rehab Mohamed Atta El-Desoukey, Microbiology and Immunology Department, National Research Center, Giza, Egypt / Shaqraa University, KSA.

Received: December 08, 2016; Published: January 04, 2017

Abstract

Since the beginning of human civilization many herbs have so far been used for the treatment and management of various ailments. Spreading of bacterial resistance against antibiotics is consider one of the common problems in the medical world, so one of the most important steps in microbiological researches is to find a new antimicrobial compound with minimal side effects. One of wild herbs was *Panicum turgidum* commonly known as Thummam, which was used as animal grazing herb especially camels. Due to that it seems that this plant may contain considerable antimicrobial capacity. So the aim of this study is to investigate the antimicrobial activity of *Panicum turgidum* aqueous extracts on some medically important animal pathogens and to determine some phytochemical compounds. Hot and cold aqueous extract of *Panicum turgidum* were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms (*Staphylococcus epidermis*, *Escherichia coli*, *Streptococcus pyogens*, *Pseudomonas aerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Candida albicans*) by agar well diffusion method. The cold aqueous extract showed high antifungal effect against *Candida albicans* in addition to significant antibacterial activity of hot aqueous extract against only *Streptococcus pyogens*. While the extracts did not show any significant antibacterial activity against the other bacterial strains. Also, phytochemical compound of aqueous extract was determined, results of the chemical tests explain the extracts of *Panicum turgidum* contain alkaloids, flavanoids, tannins and saponin compounds. So it could be concluded that the *Panicum turgidum* extract possess remarkable antifungal activity against *Candida albicans* and significant antibacterial effect against *Streptococcus pyogens* and to be introduced as an alternative to chemical antimicrobial drugs, is required wider investigation.

Keywords: Natural Antimicrobial; *Panicum*; New Antifungal; Thummam; Grazing Herbs

Introduction

Microbes are the most common cause of infectious diseases which participate in about half of the deaths in animals. In addition to the major cause of morbidity and mortality in many developing countries which mainly due to diarrhea, which are most commonly due to *Escherichia coli* (*E. coli*) and *Salmonella* spp [1,2]. Also Staphylococci is considered the main cause of subclinical mastitis caused by intramammary infections common in dairy cows [2,3]. Diarrhea in neonates are associated mainly with several enteropathogens. Their relative prevalence varies geographically, but the most prevalent infections in most areas are *Escherichia coli*, rotavirus, corona virus, and *Cryptosporidium parvum*. More than one of these etiological agents are commonly associated leading to neonatal diarrhea outbreaks [2,4]. Also systemic fungal infections due to *Candida albicans* which play an important role as a cause of morbidity and mortality [2,5]. *Bacillus cereus* was identified as an in frequent abortigenic agent in cattle. Necrotizing placentitis with no or sporadic lesions in fetal tissues [2,6]. *Klebsiella pneumonia* is a common cause of clinical mastitis in dairy cattle and the main source are wood products [2,7].

Citation: Rehab Mohamed Atta El-Desoukey. "Phytochemical and Antimicrobial Activity of *Panicum turgidum* (Thummam) as a Grazing Herb against Some Animal Pathogens". *EC Microbiology* 5.1 (2017): 22-29.

In the present time ,interesting in the using medicinal plants to dominance the growth of the pathogenic microorganisms, due to a large number of chemical antibiotic were costly and exhibit side effect therefore, the award people are turning towards herbal antimicrobial [2,8], and the synthetic medicament have been banned in the world because of their undesirable attributes such as high and acute, long degradation periods [2,9] bacteria resistance of antibiotics that may occur due to, a) active transport system deficiency which means that Neomycin enters bacterial cells is dependent on the presence of oxygen in the environment, which explains the natural resistance of anaerobic bacteria, b) reduction of membrane permeability, which makes the antibiotic concentrations do not perform active in bacterial cells [2,10]. Also, antibiotics had residues in animal products [2,9]. For this, trying to find alternatives to antibiotic. In accordance to what had been mentioned by World Health Organization (WHO), more than 80% of the world's population depend on traditional medicines for their requirements of primary health care. The plants medicinal value depends on its content from chemical substances which produce physiological action on human body. The most important of these bioactive compounds of plants include alkaloids, flavonoids tannins and saponins. An effective approach of the phytochemical research generally in the discovery of new anti-microbial from higher plants [10,11].

For instance, *Panicum turgidum* commonly known as Thummam present as a perennial bunchgrass, growing in dense bushes .The stems are long-jointed, hard and polished, with few leaves, This is a drought and salt-tolerant species [11-13] and used for flour, fodder, thatch and erosion control [14]. Camels and donkeys can eat the leaves and shoots of this grass in the dry state because it is palatable to livestock [15].

Panicum turgidum is the best forage and pasture grass in the tropics [16,17]. Anti-diabetic and antibacterial activities of Ethanol leaf extract showed against clinically important microbial pathogens [17,18]. In Nigeria the plant (leaf) is used in treatment of various diseases such as malaria, infections, rheumatism pain, inflammation and diabetes, traditionally [17,19]. The activity of the leaf extract as anti-diabetic, Antiplasmodial and analgesic activities have also been reported [17]. In addition to the activity of the ethanolic leaf extract as anti-inflammatory and antipyretic [17,20].

Doss., *et al.* [2011] reported antibacterial activity of, *Panicum turgidum* leaf on selected bacterial strains when compared with the standard drug ciprofloxacin. It has also been reported its anti-diabetic and antibacterial activity against some pathogenic bacteria. But there is no report about its antifungal activity up till now [11,18].

So the aim of this study is to investigate the phytochemical and antimicrobial activity of, *Panicum turgidum* aqueous extract as a grazing herb against some medically important animal pathogens (*Staphylococcus epidermis*, *Escherichia coli*, *Streptococcus pyogens*, *Pseudomonasaerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Candida albicans*) by agar well diffusion method.



Figure 1: Desert grass *Panicum turgidum* (local name Thummam) [21].

Materials and Methods

1. Collection of plant materials: *Panicum turgidum* (Thummam) used in this study were obtained from local farm in Alquieiya, KSA, 2016.

2. Aqueous extraction: Distilled water was boiled, dried plants were added to the water and left to cool. Then were mixed by the blender and filtered to get clear aqueous extracts. The extracts were kept at 4°C until to be use. But the hot aqueous extract has been prepared directly after boiling and filtration.

3. Preparation of inoculums: The inoculums (bacterial strains and fungi) were isolated from large animals and poultry farms on the outskirts of Cairo .The strains of bacteria (*Staphylococcus epidermis* , *Escherichia coli* ,*Streptococcus pyogens*, *Pseudomonasaerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumonia*) and fungi (*C. albicans*) were inoculated Sabaroud dextrose agar (SAB) (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) and nutrient broth (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) for overnight at 37°C for bacteria and 25°C for fungi.

4. Antimicrobial screening: The agar well diffusion method was used for the determination of antibacterial activity of *Panicum turgidum* (thomam) aqueous extracts by using bacterial isolates taken from animals and poultry (*Staphylococcus epidermis*, *Escherichia coli*, *Streptococcus pyogens*, *Pseudomonasaerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumoniae*) and fungi (*C. albicans*) to evaluate its effects on the isolated bacteria. Loopfull growth from bacterial isolate was inoculated into nutrient broth incubated at 37°C for 18 hours. The dilution of bacterial suspensions with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to get a uniform suspension containing 1.5×10^8 CFU / ml. Muller- Hinton agar was inoculated with 0.1ml of bacterial inoculum .Using cork borer, wells were made on the cultured media. The aqueous extracts were considered as the 50% concentration. Then, 0.1ml of aqueous extracts were added to wells, then the plates left for 30 min in refrigerator at 4°C, thereafter, they were incubated at 37°C for 24 hrs. The activity of aqueous extracts was determined by measuring the diameter of inhibition zone in millimeter. All experiments were duplicated. Ciprofloxacin (10 µg) and penicillin (10 µg) used as positive control while distilled water (100 µg) used as negative control for antibacterial screening aqueous extract. Nystatin (10 µg) was used as positive control while distilled water (100 µg) used as negative control for antifungal screening .All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) [22].

5. Phytochemical Tests

- 1. Tannins Test:** A modified methods stated in [23] was used to be presented of tannins on the extracts, Afew drops of Ferric chloride reagentwere added for 3 ml of extract. A blueblack color refereed to the present oftannins.
- 2. Alkaloids Test:** A fewdrops of Marqus reagent (prepared from mixing 0.5 ml of Formaldehyde with 5ml of concentration H_2SO_4), added tothe 5 ml of extract. Turbidity refereedto the present of alkaloids [24].
- 3. Saponins Test:** 3 ml of extract was added to the 2 ml of Ferric chloride, a white residue to be formedas evidence to the present of Saponins [25].
- 4. Flavonoids Test:** Flavonoids test were implement in conformity with [25]. 2ml of extract mix with Alcoholic KOH (0.5 mol.), a yellow color as proofed to the present of Flavonoids.
- 5. Glycosides Test:** 0.5g of grinded Thomam was dissolved in 2ml of glacial acetic acid containing one drop of Ferric chloride solution, and then under laid with 1 ml of concentration H_2SO_4 a brown ring indicated the present of Glycosides [26]. All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company).

Results

This investigation of antimicrobial activity was performed on hot and cold aqueous extracts of *Panicum turgidum* (Thomam) as shown in (Table 1). The screening step in the preliminary study for antimicrobial activity was done using the Agar well Diffusion Method. The diameter of the clear zone indicated the inhibition activity. The cold aqueous extract showed high antifungal effect against *Candida albicans* in addition to significant antibacterial activity of hot aqueous extract against only *Streptococcus pyogenes*. While the extracts did not show any significant antibacterial activity against the other bacterial strains. Phytochemical screening indicated that the aqueous extract most abundantly contained alkaloids, flavonoids, tannins and saponin, while no glycoside was yielded from extract as shown in Table (2).

Type of extract		Aqueous extract		Ciprofloxacin	Bacitracin	Nystatin	Distilled water
Type of microorganism		Hot aqueous extract	Cold aqueous extract				
G-Ve	<i>E coli</i>	0	0	20	17	0	0
	<i>Salmonella</i>	0	0	30	22	0	0
	<i>Enterococcus</i>	0	0	35	30	0	0
	<i>Ps.aerogens</i>	0	0	30	22	0	0
G+Ve	<i>Bacillus</i>	0	0	34	36	0	0
	<i>S.epidermis</i>	0	0	25	33	0	0
	<i>S.pyogenes</i>	18	0	0	30	0	0
	<i>K.pneumonia</i>	0	0	38	39	0	0
Fungi	<i>C.albicans</i>	0	18	0	0	16	0

Table 1: Antimicrobial activity of *Panicum turgidum* (Thomam) aqueous extract against some animal pathogen in (mm).

Plant extracts Phytochemical tests	<i>Panicum turgidum</i>
Flavonoids Test	+
Alkaloids Test	+
Glycosides Test	-
Saponins Test	+
Tannins Test	+

Table 2: The phytochemical compounds in *Panicum turgidum* aqueous extracts.
+ (contain this phytochemical compound)

Discussion

Naturally occurring antibacterial compounds can be derived from plants, animal tissues, or microorganisms [2,27]. The need for developing the newer antimicrobial compounds due to the antimicrobial compounds side effects and antibiotic resistance. So the medicinal plants have been examined for newer antibiotic with potent activity [2,28]. Antimicrobial agent must suppress or destroy microbial growth; thus susceptibility of the microorganisms, penetration of antimicrobial agent to the infected site, adequate concentration of the agent, low toxicity of the host cell, lack of micro-organism development of resistance to the agent are necessary [2,29].

So the aim of this study is to investigate the phytochemical and antimicrobial activity of, *Panicum turgidum* aqueous extract as a grazing herb against some medically important animal pathogens (*Staphylococcus epidermis*, *Escherichia coli*, *Streptococcus pyogens*, *Pseudomonasaerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Candida albicans*) by agar well diffusion method.

A lot of studies involve extraction of the active component in the plants using organic solvents. But plants as used in traditionally, using organic solvent extraction for the antibacterial properties should not be done often; therefore, in the present study the commonly used plant extract are made with distilled water and tested for its antimicrobial effect against (*Staphylococcus epidermis*, *Escherichia coli*, *Streptococcus pyogens*, *Pseudomonasaerogenes*, *Salmonella typhimurium*, *Enterococcus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Candida albicans*).

All plants used in the traditional medicine mostly have moderate antimicrobial activities as reported earlier [2,30-32].

The significant antifungal action of ethanol leaf and floret extracts of *Panicum turgidum* suggests the presence of potent antifungal components [11,33] obtained similar results on *Ficus vallis – choudae* and *Detarium microcarpum*. Variation in zones of inhibition of growth of test organisms caused by the two extracts suggests host specificity of the extracts [11,34].

Considering the above results, our result showed the high antifungal effect of the cold aqueous extract of *Panicum turgidum* against *Candida albicans* in addition to significant antibacterial activity of hot aqueous extract against only *Streptococcus pyogens*. While the extracts did not show any significant antibacterial activity against the other bacterial strains.

The presence of biologically active constituents such as alkaloids, tannins, saponins and flavonoids in leaf and florets though in varying percentages might be responsible for the antifungal activity observed in the present study. Also the potency of leaf extract when compared with stem and floret could be due to high percentage of alkaloids and saponins in the extract. Alkaloids have been reported to possess antimicrobial activity against organisms such as *klebsiella pueumonia* and *Candida albican* [11]. The *Terminalia citrina* extracts tannin content causes inhibition of cell wall formation leads to death of fungi [11,35]. However, non- inhibition of *Candida albicans* and *Aspergillus flavus* suggests resistance of these fungi to the active component present in the leaf extract. Also [11] and [36] reported the antifungal activity of tannins through coagulating the protoplasm of the microorganism. Biological function of flavonoids includes protection against allergies, inflammation, free-radicals, platelet aggregation, microbes, ulcer, hetatoxins and tumors [11,37]. This may be explained by its activity against the test organisms. Also it has been reported by [11,34] that flavonoids are responsible for anti fungal activity of some other medicinal plants. The absence of alkaloids, tannins and saponins in the stem extract may be responsible for its non-activity against all the organisms tested.

In this regard, the study was performed to investigate the phytochemical contents of *Panicum turgidum*. All phytochemical tests results indicated that the aqueous extract most abundantly contained alkaloids, flavnoids, tannins and saponin, while no glycoside was yielded from extract as shown in Table (2).

Antimycobacterial properties of the herb could be due to the abundant saponins, and tannins that were found in it Table (2). Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids, and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects, and other herbivores [38].

Therefore, in order to obtain effective antimicrobial activity at low concentrations without any side effects on the health researches should focus on the optimization of purification and applications.

Also it can act as growth promoter through its effects by increasing activity of metabolic cycles because it contains Tannins (high molecular weight phenolic compounds) that have enough hydroxyl ions to bind feed nutrients (protein, carbohydrates and vitamins) and precipitate feed nutrients in tissues [39]. The bacteriostatic or bactericidal of tannin against *Staphylococcus aureus* have been reported [40]. The mechanisms of tannin antimicrobial activity can be explained briefly as follows. (i) Many microbial enzymes have been inhibited by tannin astringent property. (ii) A tannin's can cause microbial membrane toxicity. (iii) This toxicity due to metal ions produced by tannin [41].

Conclusions

So it could be concluded that the *Panicum turgidum* extract exhibited remarkable antifungal activity against *Candida albicans* and can be introduced as promising alternative natural antifungal for treating animal mycotic infection by *Candida albicans*, in addition to significant antibacterial activity against *Streptococcus pyogenes* but required wider investigation. These results supported the claimed use of this herb as a rich grazing pasture for animals. However, it is necessary to justify the safe use of these herbal drugs through the detailed fractionation, toxicity and determination of the active constituent.

References

1. Parastoo Karimi Alavijeh., *et al.* "A study of antimicrobial activity of few medicinal herbs". *Asian Journal of Plant Science and Research* 2.4 (2012): 496-502.
2. Rehab Mohammed Atta ElDesoukey. "Phytochemical and Antimicrobial Activity of *Salvadora persica* (Miswak) against Some Animal Pathogens". *Asian Academic Research Journal of Multidisciplinary* 2.6 (2015): 199- 212.
3. Thorberg BM., *et al.* "Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci". *Journal of Dairy Science* 92.10 (2009): 4962-4970.
4. Diarrhea in Neonatal Ruminants Copyright © 2010-2014 Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, N.J., U.S.A.
5. Beuchat L R and D A Golden. "Antimicrobials occurring naturally in foods". *Journal of Food Science and Technology* 43 (1989): 134-142.
6. Schuh J and Weinstock D. "Bovine abortion caused by *Bacillus cereus*". *Journal of the American Veterinary Medical Association* 187.10 (1985): 1047-1048.
7. Munoz MA., *et al.* "Fecal shedding of *Klebsiella pneumoniae* by dairy cows". *Journal of Dairy Science* 89.9 (2006): 3425-3430.
8. Abd El-Latif SA., *et al.* "Effect of feeding dietary thyme, black cumin, dianthus and fennel on productive and some metabolic responses on growing Japanese quail. Egypt". *Poultry Science* 22.1 (2002): 109-125.
9. Buchanan NP., *et al.* "The effect of a natural antibiotic alternative and anatural growth promoter feed additive on broiler performance and carcassquality". *Journal of Applied Poultry Research* 17.2 (2008): 202-210.
10. Duraipandiyan V., *et al.* "Antimicrobial Activity of Ethnomedicinal Plants used by Palyar Tribe from Tamil Nadu India". *Complementary Alternative Medicine* 6 (2006): 35.
11. Kanife UC and Odesanmi OS. "Phytochemical composition and antifungal properties of the leaf, stem and florets of *Panicum maximum* Jacq. (Poaceae)". *International Journal of Biology* 4.2 (2012): 64-69.
12. Aganga AA and Tshwenyane S. "Potentials of Guinea grass (*Panicum maximum*) as forage crop in livestock production". *Pakistan Journal of Nutrition* 3.1 (2004): 1-4.

13. M. Ajmal Khan., *et al* "Panicum turgidum, a potentially sustainable cattle feed alternative to maize for saline areas". *Agriculture, Ecosystems and Environment* 129.4 (2009): 542-546.
14. Anthelme F and Michalet M. "Grass-to-tree facilitation in an arid grazed environment (Air Mountains, Sahara)". *Basic and Applied Ecology* 10.5 (2009): 437-446.
15. "Panicum turgidum Forsk". Grassland species: Profiles. FAO.
16. Kanife UC. "Potentials of alkaloids from Panicum maximum florets infected with the fungus Tilletia ayresii in controlling uterine contraction in Sprague- dawley rats". Ph.D Thesis University of Lagos (2011): 181.
17. Ajoku GA., *et al*. "Foliar Ultra-Structure and Antimicrobial screening of the Leaf Extracts of Panicum maximum Jacq. (Family: Poaceae/ Graminae)". *Scholarly Journal of Biological Science* 4.3 (2015): 19-22.
18. Doss A., *et al*. "Antibacterial evaluation and phytochemical analysis of certain medicinal plants". *Journal of Research in Biology* 1 (2011): 24-29.
19. Antia BS., *et al*. "Antidiabetic activity of Panicum maximum". *International Journal of Drug Development and Research* 2.3 (2010): 488-492.
20. Okokon JE., *et al*. "Antiinflammatory and Antipyretic Activities of Panicum maximum". *African Journal of Biomedical Research* 14.2 (2011): 125-130.
21. http://www.floraofqatar.com/panicum_turgidum.htm
22. Mahon CR and G Manuselis. Textbook of diagnostic microbiology. W.B.Saunders, Pennsylvania (1995).
23. Chung KT., *et al*. "Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds". *Letters in Applied Microbiology* 17.1 (1993): 29-32.
24. Chung KT., *et al*. "Tannins and human health: a review". *Critical Reviews in Food Science and Nutrition* 38.6 (1998): 421-464.
25. Sasikumar JM., *et al*. "Antibacterial activity of Eupatorium glandulosum leaves". *Fitoterapia* 76.2 (2005): 240-243.
26. AlKharazgi SM. "Biopharmacological study of Artemisia herba alba". MSc. Thesis, University of Baghdad (1991).
27. Jordon MC and David JN. "Natural product drug discovery in the next millennium". *Pharmaceutical Biology* 139.1 (2001): 8-17.
28. Cordell GA. "New roots for an old science". Atta-ur-Rahmaninand Basha FZ., (Eds) Studies in Natural Chemistry. Pharmacognosy 13: Natural Bioactive Products (Part A), Elsevier, Amsterdam (1993).
29. Sundquist G. "Taxonomy, ecology and pathogenecity of the root canal flora". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 78.4 (1994): 522-530.
30. Soliman OE. "Evaluation of myrrh (Mirazid) therapy in fascioliasis and intestinal schistosomiasis in children: immunological and parasitological study". *Journal of the Egyptian Society of Parasitology* 34.3 (2004): 941-966.
31. Takazawa H., *et al*. "An antifungal compound from shitake (Lentinus edodes)". *Yakugaku Zasshi (Japanese)* 102.5 (1982): 489-491.
32. Wondill Froman. "Biblical Facts About Wine: Is It a Sin to Drink Wine?" AuthorHouse (2005): 307.
33. Adekunle A A., *et al*. "Antifungal activity of Bark Extract of Ficus Vallis – Choudae Delite – boll (Moraceae) and Detarium Microcarpum Guill-Perr. Caesalpinaceae". *Journal of Life and Physical Sciences* 2.2 (2005): 64-67.

34. Adekunle A A and Okoli S O. "Antifungal activity of the Crude Extract of *Alafia bateri* Oliver (Apocynaceae) and *Hasmanthera dependens* Hochst. (Menispermaceae)". *Hamdard Medicus* 55.3 (2002): 52-56.
35. Burapadja S and Bunchoo A. "Antimicrobial activity of Tannins from *Terminalia Citrina*". *Planta Medica* 61.4 (1995): 365-366.
36. Onadapo J A and Owonubi N O. "The antimicrobial Properties of *Trenia Gluneensis*". In: 1st NAAP Proceedings Faculty of Pharmaceutical Science, ABU Zaria, (1993): 139-144
37. Okwu D E. "Phytochemical vitamins and mineral contents of two Nigeria Medicinal Plants". *International Journal of Molecular Medicine and Advance Science* 1.4 (2005): 375-381.
38. Salama HM and Marraiki N. "Antimicrobial activity and phytochemical analysis of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt". *Australian Journal of Basic and Applied Sciences* 3.3 (2008): 2008-2015.
39. Chrispinus DKR., *et al.* "Polyphenols and minerals composition of selected browse tree species leaves native to north-western Tanzania traditional fodder banks". *Journal of food, Agriculture and Environment* 4.1 (2006): 328-332.
40. Trease GE and Evans WC. A text book of pharmacognosy. 14th ed". Bailliere Tindall Ltd.London (1996).
41. Harborne J B. "Pytochemical methods :Aguide to modern techniques of plant analysis. 2nd ed". Chapman and Hall,London .UK (1984): 288.

Volume 5 Issue 1 January 2017

© All rights reserved by Rehab Mohamed Atta El-Desoukey.