Internal Evaluation Report for Article No. 86820-PJN-ANSI

The above mentioned manuscript has been evaluated by the Internal Academic Editor of ***Science Alert***. On the basis of internal evaluation it is intimated that your manuscript falls within the scope of the journal and can be published in Pakistan Journal of Nutrition. Although the Internal Academic Editor found considerable merits in your article, they also identified some serious concerns. These concerns should be addressed in the revision of the article. Therefore, you are invited to respond to these concerns which are given below and in the main body of the text as side notes and submit your revised article on urgent basis.

Comment 1

The Internal Academic Editor has pointed out a large number of grammatical and sentence construction errors in the article. Most of the sentences are haphazard, extra ordinary lengthy and hard to understand. You please negotiate with American Journal Experts, or Bioscience Editing Solutions to provide you language editing services at competitive rates.

Non English authors may contact with American Journal Experts or Bioscience Editing Solutions to correct spelling, grammar, punctuation and word usage errors before submission or after acceptance of their manuscripts to eliminate (minimize) the chances of rejection due to poor English. Please re-submit your article after editing its language with editorial certificate for further processing.

American Journal Experts offer a 10% discount to the authors of Pakistan Journal of Nutrition. To receive this discount, authors can enter the code **SCIALERT** from the referral code box at the bottom center of the American Journal Experts (AJE) dashboard.

Comment 2

Abstract is not prepared according to the journal format and it does not provide basic content of the paper. Please note that the journal requires the Abstract to be divided into the following sections: Background/Objective, materials and methods, results and Conclusion.

Background/Objective: In this section, a brief description of the context and purpose of the study is presented.

Materials and Methods: In this section author briefly describe how the study was performed and which statistical tests were being used.

Results: Author presents the summary of main findings of the study.

Conclusion: In this section author states the conclusions of the study clearly and concisely.

The following example will serve to illustrate the structure of an Abstract.

Abstract

Background: The cecum of the chicken gut may be susceptible to pathogens because it is readily colonized by microbes. The lower segment of the gut is also the primary tissue that permits the invasion of microorganisms from the external environment and the cloaca. Mucins, which are composed of glycoproteins, play significant roles in forming the barrier against infection on

the mucosal surface. **Objective:** The aim of this study was to determine the differences in the mucosal barrier of the lower segment of the gut between Indonesian naked neck chickens and normal feathered chickens. **Methodology:** The lower segments of the gut (rectum, colon and cecal tonsil) of Indonesian indigenous naked neck chickens and normal chickens were collected. The expression of the mucin 2 gene in the gut mucosa was analyzed by reverse-transcription-polymerase chain reaction (RT-PCR). The localization and molecular sizes of the mucosal glycoproteins were analyzed by Western blot. Wheat Germ Agglutinin (WGA) and jacalin lectins were used for Western blot analysis. **Results:** The mucin 2 gene was expressed in the mucosal gut of the rectum, colon and cecal tonsil in both naked neck chickens and normal chickens. Western blot analysis showed a single band for both WGA and jacalin from the mucosal gut of the rectum, colon and cecal tonsil in both naked neck chickens and normal chickens. **Conclusion:** These results suggest that the mucin 2 gene and glycoproteins containing WGA and jacalin positive sugars cover the surface of mucosal gut in both naked neck chickens and normal chickens, most likely to form a mucosa barrier.

Comment 3

References are not cited in the text according to the format of the journal. References must be cited in the text in superscript digits at the end of sentence or paragraph before punctuation or full stop[1]. In case of two or more references, separate the superscript digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6]. For more information, take a look at the following examples:

Lighting levels in the U.S.A are often kept below 10 lux in commercial broiler houses while the Commission of the European Communities[5] restricts the use of low intensities (<20 lux). Low light intensity is used to increase feed efficiency and keep birds calm[6,7]. High intensity light has been observed to lower body weight and poorer feed conversion[8-10]. However, numerous other studies have shown that intensity of light has little effect on food intake, final body weight and feed conversion[8,11-14].
Rozenboim *et al*.[15] and Zimmermann[16] observed that there were differences between even types of fluorescent bulbs and incandescent bulbs in body weights of broilers. Mendes *et al*.[17] found that birds raised under LEDs performed better overall than birds reared under CFLs.

Comment 4

Significance Statement (120 words maximum) (Compulsory)

A statement about the significance of this research work should be included in the manuscript. The significance statement should provide the novelty aspect and significance of this research work with respect to the existing literature and more generally to the society. It should be a short summary which describe what this paper adds to and what was already known.

Start this statement with the following words:

This study discover the ---------- that can be beneficial for ……

And the last sentence of this statement could be such as:

This study will help the researcher to uncover the critical areas of ---------- that many researchers were not able to explore. Thus a new theory on ---------- may be arrived at.

A Model Significance Statement:

This study discovers the possible synergistic effect of vitamin E, calcium, and vitamin D combination that can be beneficial for osteoporosis-induced ovariectomized rats. This study will help the researcher to uncover the critical area of postmenopausal bone loss

that many researchers were not able to explore. Thus, a new theory on these micronutrients combination, and possibly other combinations, may be arrived at.

Comment 5

Statement on Conflicts of Interest

A statement on conflicts of interest should be included in the manuscript. Either mention: 'none declared', or specify the authors' financial or other interests which should be known to the readers.

Comment 6

Kindly refer to the list of References - References are each must be numbered, ordered sequentially as they appear in the text.

Necessary changes should be made at first and author should send the final draft of this manuscript to American Journal Experts for editing.

You are requested that please modify your article according to the above instructions and according to the side notes given below in the text and re-submit it as early as possible for further processing.

Phytochemical Screening and in vitro Antimicrobial Effect of the Ethyl acetate Extract of silages waste of orange *(Citrus sinensis)*

[1]Ucop Haroen, [1]Agus Budiansyah and [1]Nelwida
[1]Departement of Nutrition and Animal Feed Science Technology
Faculty of Animal Science, Jambi University, Jambi
Jl. Jambi - Ma. Bulian KM 15, Mendalo Darat, Jambi.
email: ucop_haroen@unja.ac.id

ABSTRACT

This study was done to determine phytochemical compounds and antibacterial activity of ethyl acetate extract of silages waste of orange *(Citrus sinensis)*. Extract was applied at 250, 500, 750 and 1000 ppm, then fermented for 28 days. Each treatment was conducted on four replications. Samples were put in a jar as a silo in anaerobic condition. At the end of fermentation phytochemical screening. Data were analyzed using variance analysis of completely randomized design.

The result showed the ethyl acetate extract of silages waste of orange, revealed the presence alkaloid, flavonoid, steroid, triterpenoid, fenolic, saponin, cumarin. The antibacterial activity of the ethyl acetae extract of silages waste of orange, was assayed using Disc method and MIC *(Minimum Inhibition Concentration)*.Test microorganism were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella tyhi* and *Bacillus subtilis*. The extract inhibited the growth of all the tests organisms at different concentrations. Zone of inhibition for *E. coli* ranging 9.75±0.00mm to 16.75±0.14 mm, *S. aureus* ranging 8.00±0.23mm to12.50±0.24 mm, *S. typhi* ranging 8.50±0.24mm to 11.75±0.00mm and *B. Subtilis* ranging 7.75±0.11mm to 11.75±0.12mm. Minimum Inhibitory Concentration (MIC) for *E.coli* ranging 38.72±0.23% to 59.54±0.23%, *S.aureus* ranging 15.08±0.54% to 23.25±0.59%, *S.typhy* ranging 10.46±0.12% to 19.65±0.02% and *B. Subtilis* 9.64±0.45% to 11.28±0.44%. The extract showed varied inhibitory activities against the entire organism studied. The study has justifed the ethyl acetate extract of silages waste of orange use for the inhibition activities antibacterial, especially microbial culture of the Gram-positive strains *S. aureus*, *B. Subtilis* and the Gram negative strains *E. coli* and *S.typhy*.

Keywords *: orange waste extract, silages, phytochemical, inhibitory bacteria, ethyl acetate*

Comment [WU1]: Please note that the journal requires the Abstract to be divid into the following sections: Background/Objective, materials and methods, results and Conclusion.

Introduction

The use plants for medicines is an ancient practice common to all societies especiallly the Indonesia society. Plants show enormous versatility in synthesizing complex materials which have no immediate abvious growth or metabolic fuctions. These complex materials are called secondary metabolites (Akinmoladum *et al*., 2007). Plants secondary metabolites have recently been raferred to as phytochemicals. Phytochemicals are naturally accurring and biological active plant compounds that have potential bacterial growth inhibiting activities. It is bellived that phytochemical compounds may be effective combating or preventing disease due to their antioxidants effect and bacterial growth (Miller *et al.*, 2004). Antioxidants protect other molecule (in vivo) from oxidation when they are exposed to free radicals and oxygen species which have been implicated in the etiology of many disease and in food deterioration and spoilage species.

Comment [WU2]: References must be cited in the text in superscript digits at t end of sentence or paragraph before punctuation or full stop[1]. In case of two more references, separate the superscri digits by comma[1,2,6]. Moreover, If there are more references but in continuous numbers then use dash between superscript digits[2-6].

Oranges (*Citrus sinensis*) are one of the most important and oldest horticulture products in many tropical and subtropical areas. Orange waste is a primary by product produced by the fruit processing industry and attempts have been made to use orange waste extracts as natural antibiotic and *feed additive* for animals (Callaway *et al.*, 2008;

Haroen *et al*., 2013). As bioactive compounds are a valuable source of flavonoids, steroids, triterpenoids, phenolic, saponin, cumarin and vitamin C (Miller *et al*., 2008) and especially limonoids compounds (Haroen *et al*., 2013). Recent phytochemical studies on the silages waste of orange juice revealed the presence of flavonoids, steroids, triterpenoids, phenolic and saponin. The orange waste extracts are rich in nutrients and contain many phytochemicals, they can be efficiently used as antibiotics or as feed supplements too. Sice there is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative feed supplements that is regarded as safe. The orange waste extracts if proved to have antibacterial activity, they can be also used in same food industry which generate large pell waste as a food preservative.

This study was aimed to focus on waste minimization in fruit processing industry. The combined efforts of waste minimization during the production process and recovery of valuable product substantially reduce the amount of waste, as well as boost the environmental scientific basis for the use of pells of citrus fruits by determining the chemical constituents as well as qualitative analysis of crude phytochemicals. The present work has been designed to evaluated the antibacterial potential and phytochemical compounds of the extract from the orange *(Citrus sinensis)* waste silages. Culture of the Gram-positive strains *S. aureus*, *B. Subtilis* and the Gram negative strains *E. coli* and *S. typhy* were prepared on nutrient agar plates.

Materials and Methods

Plant material:

The plants used in this study were *Citrus* sinensis (common name: sweet orange). The waste were collected from the local fruit juice shops. Affter colection the orange waste were shade dried at oven temperature (45-50 ^{o}C) to constant weight over a perior of 5 days. Each of the plant parts were coarsely powdered using a mortar and pestle and were futher re duced to powder and were used to prepare the products, 2 kg orange waste then mixture was stirred with the addition molases and rice bran and then wrapped with plastic bag and stored at 28 days, samples were put in a jar as a silo in anaerobic condition. The dried silage waste of orange was pulverized orange waste were kept separately in an air-tight cellophane bag until used.

Preparation of extracts:

Waste silages mashed with orange using a mortal and pestle as much as 2 kg then at room temperature. Then filtered to obtain the extract, the extract was concentrated using a rotary evaporator at a temperature of 40 ^{o}C. Exactly 700 g each of the pulverized plant was macerated successively in ethyl acetate 95% for 36 h each. The mixture were then filtered under vacum and the filrates concentrated using a rotary evaporator. The ethyl acetate concentrate was evaporated to dryness in a water bath. The ethyl acetate extract of silages waste orange at 250, 500, 750 and 1000 ppm concentrations 10, 20, 30, 40 and 50% aqueous ethyl acetate extract of silage waste of orange were introduced into each of the petridishes. Coleridin (antibiotic) only served as control . The contents of the petridishes were incubated at a temperature of 28 ^{o}C. The petrdishes were covered with glass to prevent evaportion. Each treatment was replicated four times in Completely Randomized Design. If necessary, a Duncan´s multiple range test was applied to compare differences between means Steel and Torrie (1997).

Comment [WU3]: Describe the Statistical Analysis at the end of the Materials and Meth section.

Comment [WU4]: What probability was use to decide the level of significance?.

Aqueous Extraction

The method of Dupont *et al*. (2005) was adopted for extraction with little modification. Briefly, 15g of the powdered plant were soaked separately in 200 ml of

distilled water at ambient temperature for 24 hour under shaking condition at 130 rpm. The extract was then filtered using Whatman filter paper No 1. Each extracts transferred to glass vials and kept at 4 ^{o}C before use.

Preliminary phytochemical analysis (qualitative test)

The powered plant parts as well as the extracts were subjected to preliminary phytochemical screening following the methodology of Sofowora (1994), Harborne (1998) and Kokate (2001).

Test for alkaloids: Two milliliter filtrate was mixed with 1% HCl and about 6 drops of Mayor's reagents. A Creamish or pale yellow precipitate indicated the presence of respective alkaloids.

Test for steroids: Two milliter of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The color changed from violet to blue or green in some sample indicating the presence of steroids.

Test for flavonoids: Two milliliter filtrate was added to concentration HCl and magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids.

Test for saponins: Froth test for saponins was used. 1 g of the sample was weighed into a connical flask in which 10 ml of sterile distiled water was added and boiled for 5 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml, of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 sec. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for terpenoids (Salkowski test): Five milliliter of each extract was mixed in 2 ml, of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. Areddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for phenolic compounds: Most of the water-methanol phase were removed with a pipette into a small test tube, then added $FeCl_3$ reagent the formation of blue/purple indicate the content of phenolic compounds.

In vitro testing of extracts for antibacterial activity

Antibacterial Activity Assay:

Antibacterial activity of ethyl accetate extract of silages waste of orange juice was determined by Disc diffusion and broth dilution method (Long *et al.*, 2003). Nutient Agar (NA) and Muller Hinton broth (MHB) were used for the tests. Overnight culture were grown at 37 ^{o}C in MHB. About 2 ml of test organisms was aseptically injected into sterilized plates shaken to spread round the plates. Sterilized nutrient agar was poured on top of the test organisms aseptically after it was cooled to about 45 ^{o}C. It was shaken immediately for even distribution of the test organisms. The nutrients agar was allowed to solidify sterilized cup borer of 8 mm diameter was used to make wells on the solidified agar into which 0.5 ml diluted ethyl accetate extract of silage waste of orange of the sample were aseptically introduced using pipette under strerile conditions. The plates were incubated at 37 ^{o}C for 48 hours under room temperature.

Zone of inhibitions were measured around each well. Discs with 250 ppm coleridin served as controls. The antibacterial activity against each test organism was quantified by determining by average diameter of the zone of inhibition around the paper discs in millimeters. The tests were performed twice and average diameters of zones were measured.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of ethyl accetate extract of silage waste of orange, was determined by serial dilution method. Stock serial dilution of ethyl accetate extract of silages waste of

orange, was subjected to fold dilutions such that concentration ranged 10, 20, 30, 40, and 50%. Again 10^6 CFU bacterial suspensions were added to the tubes. The tubes were incubated at 37 ^oC for 24 h. MIC was take as the highest dilution of the extract that inhibited the growth of the bacterial lowest concentrations of the ethyl accetate extract of silages waste of orange which inhibited the growth after a period of 24 h of incubation at 37 ^oC, were recorded as MIC.

Results and Discussion

Comment [WU5]: It is observed that results are neither compared nor contrasted properly with relevant findings from other published wo Discuss the Results in more detail.

Tabel 1. Phytochemical compounds in ethyl acetate extracts of silages waste of orange

Secondary metabolites	Reagent	Observation	Result
Alkaloids	Meyer	White mist formed	(+)
Flavonoids	Sianidin test	Orange solution	(+)
Steroids	Liebermann-burchard	Blue solution	(+)
Triterpenoids	Liebermann-burchard	Red-brown solution	(+)
Phenolic	$FeCl_3$	Solution blue/purple	(+)

Table 1. Shows the phytocemicals detected in ethyl acetate extracts of silages waste of orange. Test for alkaloids, flavonoids, steroids, triterpenoids and phenolics were positive. These metabolites compounds (alkaloids, flavonoids, steroids, triterpenoids and phenolic) are know to have curatives activity against *E. coli*, *S. aureus*, *S. typhi* and *B. Subtilis*. The antibacterial activity of ethyl acetate extract of silages waste of orange, assayed by Disc diffution method is compared with the antibiotic coleridin (controls) Table 2 and Table 3. The results of the study showed that the ethyl accetae extracts of silage waste of orange demonstrated activity on bacteria *E. coli*, *S. aureus*, *S. typhy* and *B. Subtilis*. This showed the susceptibility tests against both organisms (*E. coli*, *S. aureus*, *S. typhi* and *B. Subtilis*). The ethyl accetate extract of silages waste of orange exhibited considerable levels of inhibition against all the levels. This was found to be significantly lowers compared to coleridin (antibiotic) against *E. coli*, *S. aureus*, *S. typhi* and *B. subtilis*. This suggestive of the presence of some phytochemicals compounds in the extracts of orange waste juice silages.

Table 2. Zone of inhibition (mm) of crude ethyl acetate extract of silages waste of orange against test bacteria on Mueller-Hinton agar medium using Disc diffusion method.

Bacteria	Zone of inhibition (mm)				
	Crude ethyl acetate extract of silages waste of orange (ppm)				
	Control (coleridin)	250	500	750	1000
E. coli	24.50^d	9.75 ± 0.00^a	10.50 ± 0.00^a	12.50 ± 0.27^b	16.75 ± 0.14^c
S. aureus	23.65^d	8.00 ± 0.230^a	10.75 ± 0.12^b	11.75 ± 0.18^b	12.50 ± 0.24^b
S. typhi	23.65^d	8.50 ± 0.24^a	10.75 ± 0.54^b	11.00 ± 0.00^b	11.75 ± 0.00^b
B. subtlis	22.67^d	7.75 ± 0.11^a	9.50 ± 0.00^b	11.50 ± 0.13^c	11.75 ± 0.12^c

Different superscripts in the same row refer to significantly different data ($P<0.05$)

Table 3. Minimum Inhibition Concentration crude ethyl acetate extract of silages waste of orange for different bacterial strains.

Bacteria	Percentage of Minimum Inhibition Concentration			
	Crude ethyl acetate extract of silages waste of orange (ppm)			
	250	500	750	1000
E. coli	38.72±0.23[a]	44.54±0.00[b]	57.15±0.98[c]	59.54±0.23[c]
S. aureus	15.08±0.54[a]	17.96±0.00[b]	18.15±0.14[b]	23.25±0.59[c]
S. typhi	10.46±0.12[a]	11.28±0.27[a]	18.15±0.54[b]	19.65±0.01[b]
B. subtlis	9.64±0.45[a]	10.05±0.76[a]	10.46±0.27[a]	11.28±0.44[a]

Different superscripts in the same row refer to significantly different data (P<0.05)

Ethyl acetate extract of silages waste of orange orange showed a significant antibacterial activity against all the test organisms. Ethyl acetate extract of silages waste of orange levels 1000 ppm showed a very good antibacterial activity when compared to ethyl acetate extract of silages waste of orange 250, 500 and 750 ppm. Ethyl acetate extract of silage waste of orange level 1000 ppm showed a maximum zone of inhibition against *E. coli* (16.75 mm) followed by *S. aureus* (12.50 mm), *S. typhi* (11.75 mm) and *B. subtilis* (11.75 mm) whereas the Ethyl acetate extract of silage waste of orange level 250 ppm did not show such high antibacterial activity. This antibacterial activity may be indicative of broad spectrum phytochemical compounds. In case of ethyl acetate extract of silage waste of orange levels 250 ppm and 500 ppm showed more or less the same antibacterial activity all the test organisms. Ethyl acetate extract of silage waste of orange levels 250 ppm all the test organisms showed very less antibacterial activity, when compared to other treatments. Ethyl acetate extract of silage waste of orange level 250 ppm did not show any significant effect against all the tested strains except all the test organisms. This show that ethyl acetate extract of silage waste of orange level 250 ppm has the capability of zone inhibition very less the same antibacterial activity all the test organisms. This it indicate that different ethyl acetate extract of silage waste of orange level may have diverse antibacterial agent that has different modes of action or the bacteria may have a special metabolism to overcome or adapt its activity. Ethyl acetate extract of silage waste of orange level 1000 ppm proves to be a good for the inhibition of anti-bacterial agents from to other teatments as it has shown better as antibacterial activity relating that higher zone inhibition means high concentration of single or variety of phytochemical and there fore high antibacterial activity. This statement can be validated as ethyl acetate extract of silage waste of orange level 1000 ppm has show highest zone inhibition a well antibacterial activity. Haroen *et al.* (2013) reported antibacterial activity screening of crude (limonoid-ethyl acetate), (limonoid-methanol) and (limonoid-n-hexane) of orange juice waste with concentration 250 ppm were test showed the zone inhibition in millimeter (11.75 for the bacterial *E. coli* and 10.25 for the *S. enteridis*) than limonoid-methanol (8.00 and 9.00) and limonoid n-hexane (8.00 and 9.25). However, this difference may be becauce of the difference in the phytochemical composition in various part of the plant or may be also due to the concentration and the extraction method used or environmental factors or difference in the genotypes of the citrus plant used. The ethyl acetate extract of silage waste of orange level 1000 ppm higher anti-bacterial activity as that standar antibiotic used in the study. However, coleridin showed higher activity relatively.

MIC of ethyl acetate extract of silage waste of orange are shown in Table 3 respectively. The ethyl acetate extract of silage waste of orange showed significant activity. The hydrocarbon components either remain on the surface of the medium or evaporate (Griffin *et al.*, 2000). That could be the reason for the better results obtained

by the microdilution method. Use of small volumes of the test substance and growth medium (Sokovic *et al*., 2007). The difference in the antibacterial activity with the source different concentration. Solvent has proven that not all phytochemical that are responsible for antibacterial activity are soluble in a singel solvent. The preliminary phytochemical investigation revealed the presence of various constituents of orange waste. The results are shown in the Table 1.

Comment [WU6]: What are the implications, applications, recommendations and limitations of the study? State clearly at the end of the Discussion section

Conclusions

Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways, yielding new products and meeting the requirements of essential products required in human, animal and plant. Conclusion of the bioactive compounds contributing to the antibacterial activity.

Acknowledgement

The authors are very grateful to Directorate General Strengthening Research and Development Ministry of Research, Technology and Higher Education, Republic Indonesia that was Funded this experiment by Hibah Fundamental Project 2017.

References

Comment [WU7]: References are each must be numbered, ordered sequentiall as they appear in the text

Akinmoladun. A. C., Ibukun. E. O., Afor. E., Obuotor. E. M., Farombi. E. O. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of ocimum gratissimum. Sci. Res. Essay. (2) pp (163-166).

Callaway. T. R., J. A. Carrol. J. D. Arthington., C. Pratt and T. S. Edrington. 2008. Citrus Products Decrease Growth of E. Coli O157: H7 and Salmonella Thyphimurium in Pure Culture and in Fermentation with Mixed Ruminal Microorganisms in vitro. Foodborne Pathog. Dis (5): 621-627.

Dupont. J., C. S. Consorti., P. A. Z. Suarez. (2002): Organic Syntheses. John Wiley, Chichester . (79) pp: 236.

Griffin. G. S., L. J. Markham., N. D. Leach (2000). An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. Journal of Essential oil Research (12): 149-255.

Harborne. J. B and A. J. Harborne. (1998). Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Kluwer Academic Publishers London. UK.

Haroen. U., Y. Marlida., Mirzah., A. Budiansyah. 2013. Extraction and isolation phytochemical and antimicrobial activity of limonoid compounds from orange waste juice. Pak. J. Nutr (12): 730-735.

Kokate. C. K. (2001): Practical Pharmacognosy. Vallabh Prakashan. pp. 218.

Long. H. H., N. Furuya., D. Kurose and Y. Takanami. 2003. Isolation of endophytic bacteria from salanum sp. And their antibacterial activity against plant phatogenic bacteria. J. Fac. Agr. Kejushu. Univ. 48(12): 21-28.

Miller. E. G., J. J. Peacock. T. C. Bourland. S. E. Taylor and J. M. Wright. 2008. Inhibition of oral carcinogenesis by citrus flavonoids. Nutr. And Cancer. (60) 69-74.

Miller. E.G., J.L. Porter, W.H. Binnie. I. Y. Guo and S. Hasegawa. 2004. Futher studies on the anticancer activity of citrus limonoids. J. Agric. Food Chem. (52) 4908-4912.

Sofowora. E. A., (1994). Medicinal plants and Traditional medicine in Africa. John Wiley and Sons Ltd.

Sokovic. M., D. Ptar., Marim., D. Brkic., J. L. Leo., D. V. Griensven (2007). Chemical composition and antibacterial activity of essential oil of ten aromatic plants against human phathogenic bacteria. Global Science Books Food. 1(1).

Steel. R. G. D., H. J. Torrie 1997. Principles and Procedures of Statistics. A Biometrical Approach 3rd Edition McGraw-Hill Series in Probability and Statistics