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Phytochemicals, Tannins Characterization and Antimicrobial Analyses of Dried Carrot Greens Extracts

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Abstract

In spite of the richness in nutritive values, minerals and vitamins content of chlorophyll which is highly contained in carrot greens, these greens are usually cut and thrown away as waste, these underutilizations of the carrot greens instigated the investigation for biological compounds of medicinal importance. This research is focus on the investigation of tannins in carrots greens and its biological activity, via extraction in 70 % acetone and partitioning in diethyl ether and distilled water. The extract was subjected to phytochemical screening, thin layer chromatography (TLC), column chromatography (CC) and antimicrobial activity test. The isolation of the tannins was carried out by preparative thin layer chromatography (PrepTLC). The isolated tannin was further analysed by TLC using ethylacetate-ethanol-water (8:2:1.5) as solvent system along with standard tannins, high performance liquid chromatography (HPLC) and fourier tranform infra-red spectroscopy. The plant extract gave a percentage extract of 5.45, 2.24 of aqueous and 3.22 of diethyl ether fractions after partitioning. The phytochemical screening of the crude gave tannins, alkaloids, flavonoids, saponins, cardiac glycosides and phenols. The TLC result of the isolated tannin revealed three (3) different tannins out of which two (2) matched with standard gallic acid and catechol according to similarity in R_f values (0.47 and 0.76 respectively). The HPLC analysis of the tannin also confirmed the appearance of three different tannins peaks eluting at retention times 2.199, 2.967 and 4.944 mins. The FT-IR supports the presence of the suspected compounds by showing broad absorptions peaks at 3348cm^{-1} for OH stretch, a medium peak at 1643cm^{-1} resulting from C=C aromatic stretch, and C-O absorption at 1211cm^{-1}. Both crude and aqueous fraction of the extract show positive activity on some gram-positive organism. The activity on *Streptococcus pneumoniae* of the tannin fraction is concentration dependant and these indicated that it can be used as remedy in respiratory tract infections such as those caused or aggrevated by *S. pneumoniae*. The appreciable quantity of flavonoids and tannins indicates they can be responsible for healings of wounds, inflamed mucous membranes, venous ulcers and exhibit radical scavenging activities.

Keywords: Phytochemicals, Antimicrobial, Carrot Greens, Daucus Carota L, Acetone Extract and Tannins

Introduction

The use of plant extracts in traditional medicine over the years has formed the basis of primary health care for majority of people living in rural and remote areas in Nigeria and third world countries [1]. Therefore, scientific researches into plants with pharmacologically active compounds such as tannins, flavonoids saponins, cardiac glycosides, alkaloids etc cannot be said to be overdone.

In many parts of the world, the cultivation of carrot (*Daucus Carota L*) is mainly for the edible root. Including Nigeria; in Plateau State where it is highly cultivated, it is for the root which is often consumed or eaten fresh, as a spice and garnish in food or sometimes even juiced.

Carrot greens are the green tops (leaves and stems) of carrots, also refers to as carrot tops. They are highly packed with chlorophyll; an excellent source of magnesium to promote healthy blood pressure as well as strong bones and muscles, and also have been noted to purify the lymph nodes and adrenal glands [2]. Carrot greens are rich in proteins, minerals and vitamins [3], research shows that it contains six times the vitamin C of the carrot itself [4]. They are also great sources of potassium and calcium which makes them capable of lowering blood pressure, support metabolism and help prevent osteoporosis. Not only that, they also contain a significant amount of porphyrins; a hormone which is known to stimulate the pituitary gland and lead to release of an increased level of sex hormone [5].

The greens of carrots can be used as a form of a poultice; when boiled with honey and lay to stand, do clean fretting sores [6], because of its antiseptic properties, it can also be juiced and used as mouth wash [7].

In spite of the richness in nutritive values, minerals and vitamins content of chlorophyll which is highly contained in the carrot tops, these vegetables are often cut and thrown away as waste littering the whole market places and the environment. The fact that these greens are thrown away instigated us to investigate them for biological compounds of medicinal importance.

This research focused on the investigation of tannins found in carrot greens and its biological activity.

Materials and methods

Plant Collection and Preparation

The plant material was collected from Farin Gada market, Jos North, Plateau State, Nigeria. It was identified as *Daucus carota L.* in the Herbarium of the Federal College of Forestry, Jos Plateau State, Nigeria, with specimen voucher number FHI 00527. The green tops were removed manually, washed with clean water to remove dirt and air dried under shade (sprayed on laboratory bench) for a period of four weeks. The dried greens were pulverized using mortar and pestle and sieved using a 1.5 mm mesh size sieve.

Extraction

Powdered sample (500 g) was homogenized in 1.5 liters of aqueous acetone (70%) and allowed to stand for 48 hrs with shaking periodically after every 4hrs [11]. Extract was decanted, filtered using What man No.1 filter paper and concentrated using rotary evaporation process. The concentrated extract was further evaporated in a water bath at 40 ^{O}C until a slurry paste of constant weight was obtained.

Solvent-Solvent Partitioning

The extract (10 g) was dissolved in 100 ml of diethyl ether and partitioned in distilled water to remove chlorophyll and non-tannins materials. The aqueous phase which contained the tannins was separated using separating funnel leaving the top organic layer which was assumed to contain no tannins. The tannin fraction was labeled (A) while the organic fraction was labeled (B). The tannin fraction (A) was concentrated for further characterization and antibacterial activity.

Phytochemical Screening of the Crude Aqueous Acetone Extract

The crude acetone extract was subjected to phytochemical test for the presence of saponins, tannins, flavonoids, cardiac glycosides, steroids, alkaloids, phenols and terpenoids using the prescribed procedures by Harbon [9], Trease and Evans [10], and Sofowora [11].

Thin Layer Chromatography (TLC) of the Tannin Fraction (A)

Thin layer chromatography plates pre-coated with silica gel GF_{254} (silica gel on aluminium support, thickness 0.05 mm) were used. The plates were carefully cut into smaller sizes of 3 cm length and 10 cm height. The TLC was performed using toluene-acetone-chloroform (6:6:1) as the mobile phase and allowed to go 90% of the height of the plate. The plates were then removed from the chamber, dried and visualized under UV light (254 nm) and iodine tank. The distance moved by the solvent, known as solvent front and the distance moved by the spot(s) were recorded and used for calculating the R_f values.

Column Chromatography (CC) of Tannin Fraction (A)

A glass column (18 mm x 400 mm) and a silica gel 60/200 mesh size were used for this work. [A piece of cotton wool placed at the bottom of the column served as a plug to support the adsorbent, compressed enough to support the packing, yet loosed enough not to hinder the solvent flow.] The column was gently packed with about 10 g of silica gel, until it became translucent and free of air bubbles. About 2 g of the extract was adsorbed on 1 gm of the silica gel and applied to the top of the column. The column was then eluted with 60 ml each, of toluene, chloroform, acetone and finally with the most polar formic acid respectively. The fractions were collected in 5 ml, and a total of 30 fractions were collected. The fractions were monitored by TLC and pooled according to similarities in R_f values into 4 fractions. Fractions were labeled appropriately as N_1, N_2, N_3 and N_4.

Spray Test for Tannins

The fractions obtained from the column chromatography were subjected to TLC. The spots on the chromatogram were directly sprayed with 1% ferric chloride solution and those that gave blue –black or green colour indicated the presence of tannins [12]. The fraction(s) which gave positive test with ferric chloride were further isolated using Preparative Thin Layer Chromatography (Prep TLC) and kept for spectral analysis.

Prep TLC of Column Fraction N_1

The fraction N_1 resulting from CC was further purified by isolation using Prep TLC. A band of N_1 was spotted on a 20 mm by 20 mm prepared prep TLC plate (of 1mm thickness). [The plates were prepared from TLC silica gel $60GF_{254}$(Merck) by dissolving 100 g in 240 ml of distilled water and the slurry sprayed on 20 x 20 glass plates; the plates were dried and activated at 105 ^{O}C for 30 minutes in an oven.] The spotted plates were allowed to dry for a minute and then dipped into a previously saturated developing chamber containing toluene-acetone-chloroform (6:6:1). When the solvent rises up to 90% of the height of the plates, the plates were then removed from the chamber and dried. The dried plates were visualized under UV light (254 nm) and the band of separation were marked. The marked band of separations were scrapped with a clean spatula and reconstituted in 70% aqueous acetone, filtered and then concentrated. The isolated tannin was then labeled NVF_1

Test for Antimicrobial Activity

Some Gram-negative and Gram-positive bacterial strains, *Escherichia coli* (G-ve), *Pseudomonas aeruginosa* (G-ve), *Staphyllococcus aureus* (G+ve) and *Streptococcus pneumoniae* (G+ve) respectively were used for detection of antimicrobial activity. They were obtained from the Standard and clinical bacterial strains stocked in Department of Pharmaceutical Microbiology Lab., Pharmacy Technology, University of Jos, Plateau State.

Invitro antibacterial activity of the crude acetone extract and aqueous fraction of tannins were studied using agar well diffusion method [13]. Briefly 15 ml of autoclaved nutrient

agar was cooled to 45 ^{O}C and mixed with 1ml of bacterial suspension (1.5 x 10^8 CFU/mL). The mixed medium was poured into eight (8) sterile petri-dishes and allowed to set. Five (5) Wells were made (four (4) around the plate with one (1) at the centre) on each petri-dishe, using sterile cork borer of 10 mm diameter. Wells around the plates were each filled with 100 µL of respective concentrations, while the well at the centre was filled with 100 µL of the standard drug (Penicillin). The plates were then incubated at 37 ^{O}C for 24 hrs. Antibacterial activity was recorded by measuring the zones of inhibition (diameter) around the well, vertically and horizontally.

Diameter of inhibition resulted from replicates measurements were expressed as mean average ± Standard Deviation (SD) and result presented in tables. The SD was calculated using the formula:

$$SD = \sqrt{((x - X)^2)/n - 1}$$

Results and discussion

Results

Table 1: Percentage Yield of Extract

Extracts	Quantity of Extracts (g)	% Yield
Crude Acetone	27.26	5.45
Diethyl ether	16.08	3.22
Aqueous (Tannins)	11.19	2.46

Table 2: Results of Phytochemicals Screening of Crude Acetone Extract

S/N	Phytoconstituents	Acetone
1	Tannins	+
2	Alkaloids	+
3	Flavonoids	+
4	Saponins	+
5	Cardiac glycosides	+
6	Steroids	_
7	Phenols	+
8	Terpenoids	_

Key - Absent, + Present,

Table 3: TLC Results of Crude Extract

Spot	R_f value
1	0.19
2	0.26
3	0.48
4	0.63
5	0.91

Table 4: Screening and Grouping of Column Fractions

Range of Fractions	Pooled Fractions	R_f of spots	Nature of Fraction
Fraction 16 - 17	N_1	Spot 1=0.18 Spot 2=0.29 Spot 3=0.48 Spot 4=0.58	Yellow with oily suspension
Fraction 24	N_2	0.76	Brown solution
Fraction 25	N_3	0.85	Brown solution
Fraction 26 - 29	N_4	0.80	Pale yellow

Table 5: TLC Results of NVF_1 and Standard Tannins

Fraction	Code	R_f Value
Tannin isolate	NVF_1	0.23, 0.46*, 0.75*
Tannic acid	TA	0.51
Pyrogallol	PY	0.68
Catechol	CT	0.76*
Phloruglucinol	PH	0.69
Gallic Acid	GA	0.47*

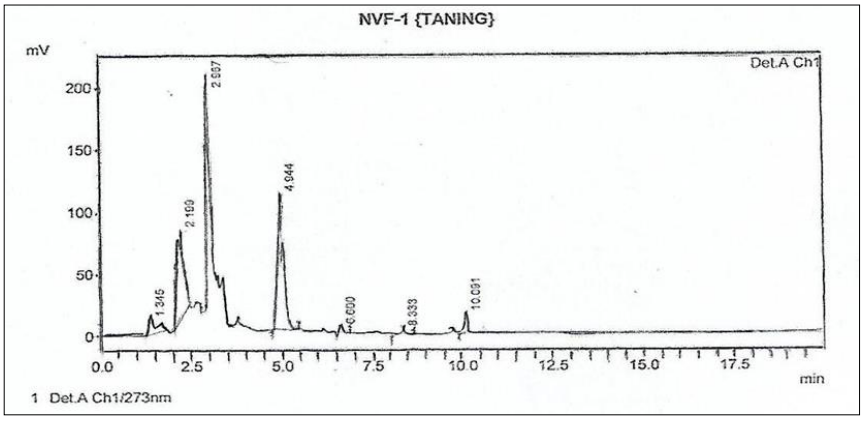

Fig 1: HPLC Chromatogram of NVF_1

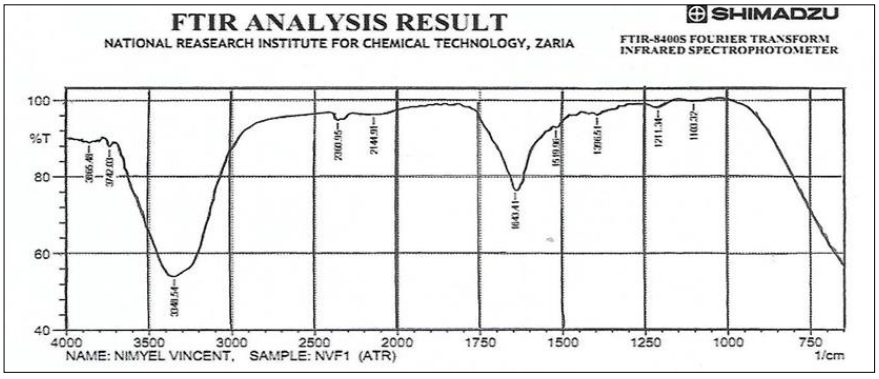

Fig 2: FTIR Result of NVF_1

Table 6: Antimicrobial Results of Crude Acetone Extract and Tannin Fraction (A)

Organisms	Zones of inhibition of different concentrations of extracts and penicillin as standard positive control drug						
	Crude Extract			Tannin Fraction (A)			Penicillin Drug
	400mg/ml	200mg/ml	100mg/ml	400mg/ml	200mg/ml	100mg/ml	STD (50mg/m)
E. coli	00.00	00.00	00.00	00.00	00.00	00.00	28.00±0.00
P. aeruginosa	00.00	00.00	00.00	00.00	00.00	00.00	35.00±0.00
S. aureus	16.50±0.25	13.75±0.31	12.25±0.31	00.00	00.00	00.00	22.00±0.00
S. pneumonia	16.25±0.10	13.50±0.30	12.50±0.30	15.75±0.10	13.00±0.00	12.25±0.10	37.75±0.10

Note: 00.00 = no activity

Discussion

Percentage Yield of the extracts

The result of the extraction of 500 g powder of the carrot greens using 70% acetone gave 27.26 g indicating a percentage yield of 5.45% for the crude acetone extract. The fraction of tannins obtained from partitioning of the crude resulted in 11.19 g with a yield of 2.24% and 3.22% yield of 16.08 g of diethyl ether.

Phytochemicals screening

The phytochemical screening of the crude acetone extract of carrot greens indicated the presence of tannins and flavonoids. Other phytoconstituents present included alkaloids, saponins, cardiac glycosides, and phenols while steroids and terpenoids were not detected. Result is summarized in Table. The alkaloids, tannins and flavonoids are known to have curative activity against several pathogens and therefore the extract could be used for the treatment of illnesses [14, 15, 16].

Tannins and flavonoids are known to treat diarrhea, especially tannins have the ability to hasten the healing of wounds and inflamed mucous membrane, haemorrhoids and varicose ulcers [17]. Alkaloids are reported to have analgesic, anti-inflammatory function and help to alleviate pain, develop resistance against diseases and endurance against stress [18]. Studies have shown that saponins found in plants have anti-tumour and anti-mutagenic properties and can also lower the risk of cancer cells [19]. They reduce the growth and viability of the cancer cells by reacting with cholesterol rich membranes of these cancer cells [19, 20]. Plants or extracts of plants containing cardiac glycosides have been used as emetics, diuretics and heart tonics. They are widely used in modern treatment of congestive heart failure and for arterial fibrillation [21]. The above narratives imply that carrot greens can be used in treatment of diarrhea, inflammation, wounds and congestive heart conditions.

Thin Layer Chromatography (TLC) of crude extract

The results for TLC analysis of aqueous tannin fraction of dried carrot greens indicated five (5) phytoconstituents as summarized in the Table 3. The spots fluoresced when viewed under UV lamp and appear as dark spots in iodine tank. This serves as a chemical fingerprint for the extracts [22]. In other word, it gives chemists a quick answer to how many components are in a mixture or crude extract which may contribute to it biological activity [23].

Column Chromatography (CC)

The result of the column chromatography (CC) of the aqueous fraction of tannin gave a total of 30 fractions; the fractions were pooled into four (4) fractions according to similarities in R_f (Table 4). Fractions 16 and 17 pooled together as N_1 had four spots: spot 1 and 2 appeared pinkish under UV, while spot 3 and 4 fluoresced. The colour became more intense when exposed to NH_3 vapour and viewed under UV; these are suspected to be phenolics. The chromatograms when sprayed with 1% solution of $FeCl_3$, fraction N_1 close to the edge turned green indicating that the tannins stay at origin while other phenolics migrated in the solvent system [24].

TLC of NVF_1 and Standards Tannins

Comparing the colour and R_f values of the standards and isolated tannin (NVF_1), similarities was observed in R_f value between gallic acid, Rf value 0.47 and catechol, R_f value 0.76 (See Table 5). Except for their colours when sprayed with ferric chloride, NVF_1 showed green confirming pseudotannin [25] while all the standards showed blue black.

HPLC Analysis

The HPLC chromatogram of isolated tannin NVF_1 (fig. I) revealed seven (7) peaks, three of which appears at a great peak height and area than the other peaks. These peaks are assumed to be the three (3) different tannins eluting at different retention times as also obtained in the TLC (table 5). The elution times for these tannins are 2.199, 2.967 and 4.944 mins, the other peaks could be due to impurities or solvents used during analysis.

FT-IR Analysis of NVF1

This result (Fig. II) showed ten (10) peaks, five (5) of which are characteristic of tannins: A broad band at 3348 cm^{-1} is indicative of OH stretch. Peak 2144 cm^{-1} can be attributed to C=C stretch. A medium peak at 1643 cm^{-1} resulting from C=C aromatic stretch, and C-O stretch of alcohols and ethers at 1211 cm^{-1} [26].

These results support the presence of catechol, the OH and C=C aromatic stretch for it is highlighted above. However, the prominent peak for C=O expected at 1725$cm^{-1}$for gallic acid is not found in the FT-IR spectrum.

Antimicrobial Analysis

The antimicrobial activities of crude and tannin fraction examined against some Gram negative and Gram-positive organisms including *E. coli, P. aeruginosa, S. aureus* and *S. pneumoniae.* The result of these analyses is presented in Table 6.

The crude acetone extract indicated activity on the Gram-positive organisms, *S. aureus* and *S. pneumonaiae* but no activity was recorded on the Gram negative. Microbiological studies have shown that Gram positive organisms are more susceptible to antibiotics than Gram negative due to the absence of outer membranes and peptidoglycan cell wall [27]. The activity of the extract is concentration dependent with high inhibition zones as 16.50±0.25 for *S. aureus* and 16.25±0.10 for *S. pneumonaie* at concentration level of 400 mg/ml of extract.

The fraction of tannins indicated activity only on *S. pneumonaiae* which is also concentration dependent with

maximum inhibition of 15.75±0.50 at 400 mg/ml concentration level. It could be said here that the additional activity as indicated by the crude is been augmented by other components synergistic activity. Other compounds such as flavonoids, alkaloids and phenolics have also been reported to have antimicrobial activity [25].

The results of the antimicrobial screening are indication that carrot greens can be used to treat or manage skin infections like abscesses, cellulitis, impetigo to blood infections like sepsis, septic arthritis, toxic shock syndrome, otitis media, sinusitis and bacteramia, life-threatening infections such as meningitis and also respiratory tract infections which are caused or aggravated by *S. aureus* and *S. pneumonaiae* [28, 29].

Summary

The phytochemicals screening of carrot greens indicated the presence of tannins, flavonoids, alkaloids, saponins, cardiac glycosides, steroids, phenols and terpenoids.

The TLC results of the crude acetone extract gave five (5) spots revealed by viewing under UV lamp and iodine tank. The fractionation of the tannin fraction yielded four (4) fractions which were pooled from thirty fractions. Fraction 16 and 17 resulting from the column chromatography were pooled together as fraction N_1. The fraction N_1 responded positively to ferric chloride test for tannins and the tannins isolated by Prep TLC and reconstituted in 70% acetone. The tannin isolate was characterized using HPLC and FT-IR. The FT-IR results confirmed the presence of tannins functional groups showing a broad band at 3348 cm^{-1} is indicative of polymeric OH stretch, C=O absorption and C-O. A medium peak at 1643cm^{-1} resulting from C=C aromatic stretch, and C-O stretch of alcohols at 1211cm^{-1}. The antimicrobial activity investigated on some gram positive and gram-negative organisms for both crude acetone and diethyl ether fraction of the tannin show activity on only gram-negative organism with inhibition zones increasing with increasing concentration.

Conclusion

In this study, the phytochemicals screening revealed an appreciable quantity of tannins and flavanoids indicating the activity in the crude and isolated tannin on some Gram-positive Organism. The isolated tannin gave three spots two of which were suspected to be gallic acid with (R_f = 0.46) and catechol (Rf = 0.75) based on R_f similarity. The significant activity of tannins on *Streptococcus pneumoniae* which is dependent on concentration, indicate that when purified can be used as remedy in respiratory tract infection.

References

1. Awosika F. Traditional Medicines as Solution to Nigerian. Health Problem in Clinical Pharmacology and Herbal Medicine. 1993; 9(3):26-31.
2. Burney P, Summers C, Chin S, Hooper R, Van Ree R, Lidholm J. Prevalence and Distribution of Sensitization to Foods in the European Community Respiratory Health Survey: A Europrevall Analysis. Allergy. 2010; 65(9):1182-1188.
3. Rubatsky VE, Quiros CF, Siman PW. Carrots and Related Vegetable *Umbelliferae*. CABI, 1999, p256.
4. http://www.carrotmuseum.co.uk/carrotops.html. Safety & Uses for Carrot Tops. Retrieved 20th March, 2016.
5. Yeager, Selene. Editors of Prevention: The Doctors Book of Food Remedies. The Latest Function on the Power of Food to Treat and Prevent Health Problems – from Aging and Diabetes to Ulcers and Yeast Infections. Rodale, 2008, p366. ISBN: 9781-1-60529-506-0.
6. http://www.nutritiondata.self.com/facts/vegetable-and-vegetable-products.html. Retrieved 21st March, 2016.
7. Hoffman D. The New Holistic Herbal (3rd ed.) Element Shaflesbury Dorset, Great Britain, SP 78 BP, 1990.
8. Hargerman AE. Tannins Chemistry, Hargerman Laboratory Manual, 1988, 1-20
9. Harbon JB. Phytochemical Methods. Chapman and Hall Ltd., London, 1973, 149-188: In African Journal of Pure and Applied Chemistry. 2009; 3(11):228-233.
10. Trease GE, Evans WC. Pharmacognosy, 11th ed., Bailliere Tindall, London, 1989, 45-50.
11. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, 1993, 191-289.
12. Evans WC. Trease and Evans Pharmacognosy, 15th edition, W. B. Sanders London, 2002, 180.
13. Velgas C, DeSouza SM, Smania EFA. Screening Methods to Determine Antibacterial Activity of Natural Products. Brazilian Journal of Microbiology. 2007; 38:369-380.
14. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial Action of Several Tannins Against *Staphylococcus aureus*. Journal of Antimicrobial Chemother. 2001; 48:487-491.
15. Kolodziej H, Kiderlen AF. Antileishmanial Activity and Immune Modulatory Effects of Tannins and Related Compounds on Leishmania Parasitised RAW 264.7 Cells. Phytochemistry. 2005; 66:2056-2071.
16. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W. Tannin inhibits HIV-1 Entry by Targeting gp 41. Acta Pharmacol Sin. 2005; 25(2):213-218.
17. Cheng HY, Lin CC, Lin TC. Anti-herpes Simplex Virus type-2 Activity of Casuarinin from the bark of *Terminalia arjuna Linn*. Antiviral Research. 2002; 55:447-455.
18. Humanson GL. "Animal Tissue Techniques" San Francisco: WH Freeman and Company, 2nd Ed, 1962, 136-137.
19. Chunfa, Huang, Carl Freter E. Cholesterol Lowering in Cancer Prevention and Therapy: Cholesterol Lowering Therapies and Drugs. IntechOpen, 2016. DOI: 10.5772/65025.
20. Olusola OE, Opeyemi I, Femi O, Esther OA. Saponins in Cancer Treatment: Current Progress and Future Prospects. Journal of Pathophysiology. 2021; 28(2).
21. www.people.vcu.edu/~urdesai/car.htm. Retrieved [20-03-2016].
22. Scott KM, Henn-Sax M, Harmer TL, Longo L, Frame CH, Cavanaugh CM. Kinetic Isotope Effect and Biochemical Characterization of Form IA RubisCO from the Marine *Cyanobacterium Prochlorococcus marinus* MIT9313 *Limnol Oceanogr* 52. 2004; 5:2199-2204.
23. Mohrig JR, Hammond CN, Schatz PF. Freeman and Company. Techniques in Organic Chemistry. 3rd Ed., New York, 2010.
24. Hargerman AE. Tannin chemistry, 2002, 110.

25. Rudi H, Syahida A, Aspollah S, Shukor MY, Elisan O. Flavonoid Analyses and Antimicrobial Activity of Various Parts of *Phaleriamacrocarpa* (Scheff.) Boerl Fruit. International Journal f Molecular Sciences. 2011; 12:3422-3431.
26. Isam Eldin Hussein Elgailani and Christiana Yacoub Ishak. Methods for Extraction and Characterization of Tannins from Acasia Species of Sudan. Parkistan Journal of Analytical Environmental Chemistry. 2016; 17(1):43-49. Doi: 10.21743/pjaec/2016.06.007.
27. Zeinab Breijyeh, Buthaina Jubeh, Rafik Karaman. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It.: Molecules. MPDI, 2020.
Doi: 10.3390/molecules25061340.
28. Tanveer Ahmad, Maria Cawood, Qumer Iqbal, Agustin Arino, Asmat Batool, Rana Muhammad, *et al*. Phytochemicals in *Daucus carota* and their Health Benefits. Foods, MDPI, 2019.
Doi: 10.3390/foods8090424.
29. Brooks LRK, George Mias I. *Streptococcus pneumoniae's* Virulence and Host Immunity: Aging, Diagnostics, and Prevention. Front, Immunol, 2018.
https://doi.org/10.3389/fimmu.2018.0366.