Original Research Paper

Volume - 7 | Issue - 8 | August - 2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 79.96

Pharmacology

SCREENING OF AQUEOUS FRUIT PERICARP EXTRACT OF *CUCUMIS MELO* AGAINST MICROORGANISMS INVOLVED IN PATHOLOGY OF VARIOUS DISEASES

Swathi P	AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam
Sravana Swathi V	Vignan Institute of Pharmaceutical Technology,Visakhapatnam
Ramkishan A	Deputy drug controller of India, CDSCO
Bhavani B	Vignan Institute of Pharmaceutical Technology,Visakhapatnam -Corresponding Author

ABSTRACT The study aimed to evaluate the effect of aqueous pericarp extract of *Cucumis melo* (APCM) for antioxidant activity and anti microbial activities against human pathogenic organisms. The antioxidant activity of APCM was carried out to assess scavenging activity against superoxide, hydroxyl, hydrogen peroxide and DPPH radicals. The APCM showed significant antioxidant activity compared with ascorbic acid. The antimicrobial activity was tested against human pathogenic bacteria and fungi. The antimicrobial activity of APCM was studied by agar well diffusion method *in vitro*. The effect of antimicrobial potential was examined o both Gram positive and Gram negative pathogens like *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae, Enterococcus faecalis.* The APCM has showed consistently significant inhibitory activity on different bacterial species tested and found the significance of antimicrobial activity. The present study revealed that the activities might be due to the presence of terpenoids and phenols in the pericarp of *Cucumis melo*.

KEYWORDS : *Cucumis melo*, Antioxidant activity, Antimicrobial activity.

INTRODUCTION:

In general health sector, infections have increased to a great extent. The antibiotic stress are common therapeutic problem upon long term antibiotic use [1]. The nature is a source of plant products with medicinal properties. And is a new source of antioxidant and antimicrobial agents with possibly novel mechanisms of action [2]. They are effective in the treatment of chronic diseases caused by oxidative stress and infectious diseases with simultaneously mitigating the adverse effects that are often associated with synthetic antibiotics and antimicrobials [3].

It is used for various ailments in Indian Traditional System of Medicine. Fruit and roots have medicinal value. The fruits are used in flatulence, leprosy, fever, jaundice, diabetes, antiobesity, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders and amentia [4,5]. It including antiulcer [6], Antihyperglycemic, Antioxidant [7], Anxiololytic, Anticancer [8,9], wound healing activity [10] and analgesic activities. All the activities might be due to the presence of chromone derivatives, triterpene, and sterols and particularly cucumol A (27-hydroxy taraxerol-3β-o1), along with α-spinasterol and D:B-friedoolean-5-ene-3-β-ol [11]. Previous the authors were reported that several pericarp extracts showed potent anti microbial activity on *Punica granatum* [12]; *Feronia limonia* [13]; *Momordica cochichinensis* [14] and *Litchi chinensis* [15].

MATERIALS AND METHODS:

Super oxide radical scavenging activity:

The assay was based on the capacity of the aqueous extract to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light- NBT system [16]. The reaction mixture contained 58 mM phosphate buffer, pH 7.6, 20µM riboflavin, 6mM EDTA, and 50µM NBT, final volume made up to 3 ml, added in that sequence. Initiated with the reaction the reaction mixture with the different concentrations was exposed to 40 volts under fluorescence lamp for 15min to intiated the reaction[17]. Immediately after illumination, the absorbance was measured at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes, with reaction mixture, above were kept in the dark and served as blanks. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_o - A_1}{A_o} \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the aqueous extract/standard. All experiments were performed in triplicate.

Hydroxyl radiacal scavenging activity:

Scavenging activity of hydroxyl radical was measured by the method of Halliwell et al., 1985 [18]. Hydroxyl radicals were generated by a Fenton reaction ($Fe3^+$-ascorbate-EDTA-H_2O_2 system), and the scavenging capacity of the extract and standard towards the hydroxyl radicals was measured by using deoxyribose method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

$$\% \text{ Inhibition} = \frac{A_o - A_1}{A_o} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

Hydrogen peroxide radical scavenging activity:

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al., 1989 [19]. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43mM H_2O_2 was prepared in 0.1M phosphate buffer (pH 7.4). The APLC of different concentrations were prepared in 3.4mL phosphate buffer were added to 0.6mL of H_2O_2 solution (43mM) and absorbance of the reaction mixture was recorded at 230 nm. All experiments were performed in triplicates. The percentage of inhibition was expressed, according to the following equation:

$$\% \text{ Inhibition} = \frac{A_o - A_1}{A_o} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample.

DPPH radical scavenging activity:

The potential of extract and AA was determined on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each concentration of extract were added to 3ml of 0.004%

MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated [20]. The percentage of inhibition was expressed, according to the following equation:

$$\% \text{ Inhibition} = \frac{A_O - A_1}{A_O} \times 100$$

Where A_0 was the absorbance of the control without a sample, A_1 is the absorbance in the presence of the sample. All tests were run in triplicate and averaged.

Test Microorganisms:

Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis, are clinical isolates collected from King George Hospital, Visakhapatnam, India.

Anti microbial assay by agar well diffusion method:

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37 ^{0}C and maintained on nutrient agar slants at 4 ^{0}C and stored at -20 ^{0}C. Inoculum of bacteria was prepared by growing pure isolate in nutrient broth at 37 ^{0}C for overnight. The overnight broth bacterial cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (Electronics India) then it was used as inoculums for fungi. The agar plates were prepared by pour plate method using 20ml of agar medium. The sterile agar medium is cooled to 45^0 C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 10^8 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at for 24 hours at 37 ^{0}C for bacteria. The diameter of inhibition zones was measured in mm using HiMedia zone reader. Ciprofloxacin (Antibiotic) was used as Standard while Solvent (DMSO) used for control [21,22].

DISCUSSION:

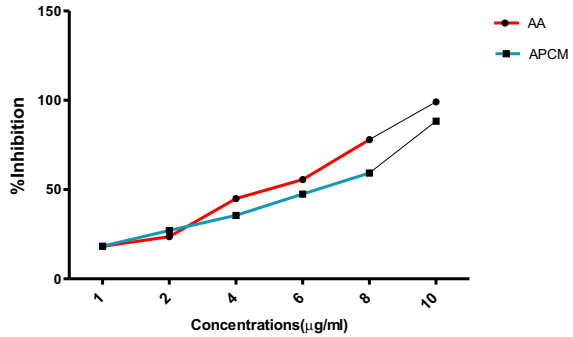
Oxidative stress plays a dual role in infections. Free radicals protect against invading microorganisms, and they can also cause tissue damage during the resulting inflammation [23]. Oxidative stress is the result of an imbalance in pro-oxidant/antioxidant homeostasis that leads to the generation of toxic reactive oxygen species (ROS), such as superoxide, hydroxyl radical, hydrogen peroxide and nitric oxide etc**.**

The superoxide radical (O_2^-)-scavenging activity of the extracts, as measured by the riboflavin- NBT-light system *in vitro*. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species [18]. The APCM showed to have significant scavenging activity against superoxide radical compared with ascorbic acid (graph 1). The hydroxyl radical can damage some macromolecules like carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation), and amino acids [24]. The evaluation of hydroxyl radicals scavenging activity has done by Fenton reaction mechanism. Hydroxyl radicals are capable to trap hydrogen atom from cell membrane and produce peroxidic reaction of lipids produced during immune action[25]. The hydrogen peroxide is involved in the formation of hydroxyl radicals. The graph 2 and graph 3 represents the effect of APCM on hydroxyl and hydrogen peroxide radical scavenging activity. DPPH is an exogenous free radical. The DPPH radical scavenging activity is extensively used in the evaluation of antioxidant activity of natural substances [26]. The degree of the discoloration indicates the scavenging potential of the antioxidant action due to its hydrogen donating ability [27]. The APCM showed to have potent DPPH radical scavenging activity like standard ascorbic acid as shown in graph 4.

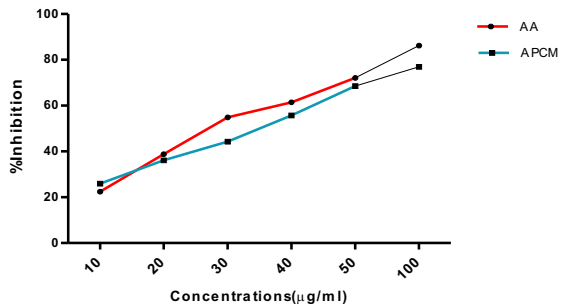
Table 1: Effect of Ascorbic acid and APCM on IC_{50} values of different *in vitro* models.

Method	Ascorbic acid	APCM
Superoxide radical	04.77±0.42	05.29±0.04
Hydroxyl radical	32.16±0.19	36.64±0.07
Hydrogen peroxide radical	05.23±0.04	03.64±0.08
DPPH radical	55.31±4.16	49.84±0.19

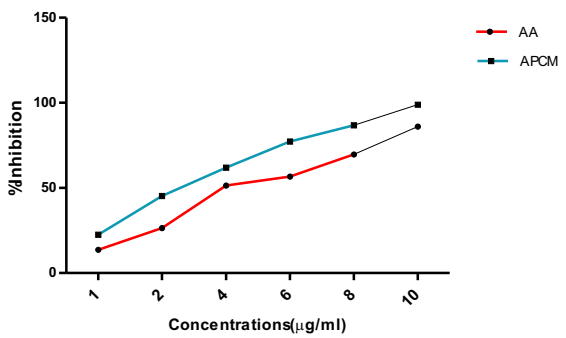
Graph 1: Effect of APCM on superoxide radical scavenging activity



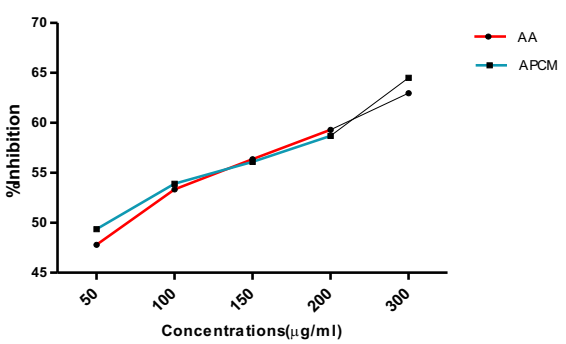
Graph 2: Effect of APCM on hydroxyl radical scavenging activity



Graph 3: Effect of APCM on hydrogen peroxide radical scavenging activity



Graph 4: Effect of APCM on DPPH radical scavenging activity



Antimicrobial studies were carried out on human pathogenic bacteria and fungi. *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis*. The APCM extract showed significant antibacterial activity against selected human pathogens. The APCM at 100 µg 10mm was the highest zone of inhibition against *Salmonella typhi*(G-ve) and against *Shigella dysenteriae* (G-ve) showed 10 mm zone of inhibition. The APCM at 100 µg concentration showed 10 mm was the highest zone of inhibition against *Vibrio cholera* (G-ve) and at 100 µg concentration of APCM extract showed 9mm highest zone of inhibition against *Enterococcus faecalis* (G+ve) . The APCM extract showed comparable antibacterial activity with Ciprofloxacin (antibiotic) (table 2).

Table 2: Anti microbial activity of APCM extract on human pathogens.

S. No	Human pathogens	Caus-ing Disease	Zone of inhibition				
			APCM 25 µg	APCM 50 µg	APCM 75 µg	APCM 100 µg	Antibiotic(30 µg)
1	*Salmonella typhi* (G-ve)	Typhoid fever	4	5	7	10	11
2	*Vibrio cholera* (G-ve)	Cholera	5	6	9	10	10
3	*Shigella dysenteriae* (G-ve)	Dysentry	4	5	8	10	13
4	*Enterococcus faecalis* (G+ve)	Gastro intestinal infections	5	6	8	9	10

The present study indicates that the antimicrobial potency of APCM against both Gram- positive and Gram- negative pathogens. The APMC also shown its scavenging activity against several free radicals involved in oxidative stress. In is concluded that the *Cucumis melo* pericarp acts as antioxidant and antimicrobial activity might be due to the presence of active phytochemicals present in it.

REFERENCES:

1. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J Agric Sci 2008; 4: 839-843.
2. Ahmad I, Aqil F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiol Res 2007; 162: 264-275.
3. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin In: Perspectives on new Crops and new Uses, eds. J. Janick, ASHS Press, Alexandria, VA, 1999; 457-462.
4. Kirtikar KR, Basu BD. Indian medicinal plant. International book seller and publisher India. 2006; 1084.
5. Chatterjee A, Prakrashi SC. The treatise on Indian medicinal plants. National institute of science and information resources, New Delhi. 1997; 5:. 114-16.
6. Gill NS, Bajwa J, Sharma P, Dhiman K, Sood S, Sharma PD, et al., Evaluation of antioxidant and antiulcer activity of traditionally consumed Cucumis melo seeds. J Pharmacol Toxicol 2011; 6: 82-89.
7. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, et al., Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J Nutr 2003; 133: 2812-2819.
8. Chu YF, Sun J, Wu X, Liu RH, Antioxidant and antiproliferative activities of common vegetables. J Agric Food Chem 2002;50(23): 6910-6916.
9. Villasenor IM, Simon MK, Villanueva AM, Comparative potencies of nutraceuticals in chemically induced skin tumor prevention. Nutr Cancer 2002; 44(1): 66-70.
10. Patil MK, Kandhare AD, Bhise SD. Pharmacological evaluation of ameliorative effect of aqueous extract of Cucumis sativus L. fruit formulation on wound healing in Wistar rats. Chron Young Sci 2011; 2: 207-213.
11. Sabrin Ibrahim. Rwaida Al Haidari and Gamal Mohamed Cucumol A: a cytotoxic triterpenoid from Cucumis melo seeds. Revista Brasileira de Farmacognosia. 2016; 26(6): 701-704
12. Eswar Kumar K, Swathi Putta, Nagendra sastry. Y, In-vitro antimicrobial and antioxidant activities of aqueous pericarp extract of Punica granatum. Journal of Applied Pharmaceutical Science. 2013; 3(08): 107-112.
13. Swathi Putta, Eswar Kumar Kilari, Nagendra Sastry. Screening of methonolic fruit pericarp extract of Feronia limonia against microorganisms involved in pathology of various diseases. Int J Phar Rea Sch, 2014;3(4):109-114.
14. Swathi Putta, Eswar Kumar Kilari. Antimcrobial activity of hydroalcoholic fruit extract of Momordica cochichinensis against some pathogenic microorganisms. Journal of Global Trends in Pharmaceutical Sciences. 2014; 5(2):1729–37.
15. Swathi Putta, Nagendra Sastry, Eswar Kumar Kilari. Screening of methanolic fruit pericarp extract of Litchi chinensis against various disease conditions. American journal of pharmacy and health sciences, 2014; 2(14):300-306
16. Beauchamp C &Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry.1971; 44: 276–277.
17. Kumaran A and Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT. 2007; 40:344-352.
18. Halliwell B, Gutteridge JMC. In Free radicals, ageing, and disease, free radicals in biology and medicine. Oxford: Clarendron Press.1985; 279-315.
19. Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogenesis. 1989;10: 1003–1008.
20. Braca A, Tommasi N, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terapotensis. Journal of Natural Products. 2001; 64: 892–895
21. Govinda Rao DY., Nagendra Sastry, D.S.V.G.K., Kaladhar. Preliminary Studies on In Vitro Antimicrobial Activity and Phytochemical Analysis of Tender Seed Coat Aqueous Crude Extract of Borassus Flabellifer Linn. Asian J Biochem and Pharm Res, 2011;3:517-523.
22. Govinda, RD. Antibacterial activity of Methanolic Seed Coat Extract of Borassus flabellifer L. Inter J Pharm Sci Res, 2011; 2(9).
23. Pohanka M. Role of oxidative stress in infectious diseases. A review. Folia Microbiol (Praha). 2013 ;58(6):503-13.
24. Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, Ortiz GG, Acuña-Castroviejo D. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res.1995; 18(1):1-11.
25. Kitada M, Igarashi K, Hirose, S & Kitagawa H. Inhibition by polyamines of lipid peroxide formation in rat liver microsmes. Biochemical and Biophysical Research Communications. 1979; 87:388-394
26. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002 Oct 31;18(10):872-9.
27. Barreira JCM, Ferreira ICFR, Oliveira MBPP a Pereira JA. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem. 2008; 107(3):1106-1113.