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Acid Neutralizing Capacity and Immunomodulatory Activity of Citrullus Colocynths

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ABSTRACT

The *Citrullus colocynthis (L.) Schard* from Cucurbitaceae family having a properties of ancient remedies on many diseases. This research work has focused on the to detect the preliminary antacid test (PAT), acid neutralizing capacity (ANC) ,buffer capacity(BC) consisting of *Citrullus colocynthis* and compared with Gelusil tablet as a standard along with this determined the immunomodulation activity. The PAT of the ethyl acetate,aqueous and alcoholic extracts of fruit were found in range of pH 3.90 ± 0.07 to 6.36 ± 0.18 and ANC located in between 24.23 ± 00 to 27.86 ± 76 mEq/gm. Further the value at which the pH of an extracts changes over period founded in between 30 min to 45 min. The investigation of immunomodulation activity carried out by phagocytosis assay of *C. albican*. This research has reached to conclusion that is the ethanolic extract yielded a greater result of ANC and aqueous extract determined higher buffer capacity comparative with standard antacid (Gelusil). Moderate immune stimulation shown by the ethanolic extract with 5 and 10 mg/ml of concentration.

Keywords: Citrullus Colocynthis, Acid Neutralizing Capacity, Immunomodulation Activity, Antacid, Buffer Capacity, Bitter Apple.

I. INTRODUCTION

*Citrullus colocynthis (L.) Schard*comes under *Cucurbitaceae* (cucurbits or gourd family). *Cucurbitaceae* is among the most important prevalent plant families in terms of the quantity and proportion of species utilized as human consumption. Citrulluscolocynthis is known as Colocynth or Vine-of-Sodom [English], Indravaruni or Brihadvani[Sanskrit], Indrayan or Ghorumba [Hindi] , Kadu-indravani [Marathi][1] It is a Wilderness bushy plant which grows in barren, droughty fields. It is native of India, Saudi Arabia, Pakistan, Shrilanka, and Africa. It is a prennial plant vine that grows tiny blooms in the desert region of India and can withstand xerophytic conditions.

Plant contains physiological components like glycosides, flavonoids, fatty acids, elatrin, alcoloids, to copherols, carotenes, albuminoids. Actively contains Cucurbitacines A,B,C,D,E,I,J,K,L having highly oxygenated and bitter in taste. Also contain yellow amorphous colocynthenin A, B, D and colorless

colocynthenin C.[2]Phytosterolin which formally present in root.

World ancient medical systems, prescribe a variety of herbal medicines which includes many plants of Cucurbitaceae family. Citrulluscolocyn this has medicinal and nutraceutical values.Citrulluscolocyn this has the traditional use in remedy for cancer[3].And also Adenocarcinoma, Lewis lung carcinoma, lymph sarcoma, Retinoblastoma, Subcutaneous and intramuscular carcinosarcoma[4].the leaves are diuretic and used to treat jaundice, asthma and more efficient as Larvicidal and Pesticidal.[5]The nomads used the seed for skin infections (Habset. Al 1984) also treat bloody diarrhea[6]the root is useful in joint agonies and is utilized remotely in ophthalmia and uterine torments. The root used in a schematic attack in children. Fruits heal quickly swelling, leukemia, and enlarged spleen, TB glands in the neck, indigestion, diarrhea, and throat illnesses.The fruit pulp is acts as strong antibacterial against many gram positive and also gram negative bacteria.[7]recently fertility activity of fruit pulp was discovered.[8]It is useful in sure pimples effectively.All fruit extract used to treatment of diabetes. It is highly showing the ach inhibitory action.[2]

The antacid having capability to neutralize the amount of acid that capability also measured as acid neutralizing capacity (ANC) of an antacid. Mainly it is a distinction between anions of stong acid and cations of strong base. An antacid is indeed a remedy that works by neutralizing more HCl in digestive juices and inhibiting the proteases, pepsin to reduce H+ in the abdomen.[9]Antacids having the different acid neutralization capacities. That can be used in dyspepsia, heartburn, duodenal ulcer. Reducing discomfort, pyloro spasms alleviation, acidic insoluble fiber digesting as well as rusting are avoided arethe main objectives of antacids. Maintain high blood phosphate level, increase uric acid solubility, diarrhea are some of the other purpose of antacid.[10]

Phagocytosis is the initiate move of defending against infectious diseases from the intrinsic immune function, also it is the interiorisation oppression of infectious agent by phagocytic cell that is preceded by pathogen slaughtering and devastation. Immunotherapy medications alter the defense program's reaction by raisingor reducing serum antibody responses.[11]

This work was therefore crated to analyze the acid neutralizing capacity with immune modulator activity of the aqueousand ethanolicextracts of Citrullus colocynthis (L.) Schard fruit

II. METHODS AND MATERIAL

Collection and extraction

Citrullus colocynthis (L.) Schard plants were collected in January from the droughty area near Solapur district of Maharashtra, India. The plant was identified by Dr. Potdar in the Department of Botany at Yashvantrao Chavan College of Science, Karad with voucher number 2.The plants had been dried. And the powder was made up of its fruits. The extraction was done by using Soxhlet's Extraction Method with ethyl acetate, ethanol and water as solvents differently. After the complete extraction by the decreasing pressure, the solvent was separated.

Preliminary antacid test (PAT)

Precise hefted quantity of a 20 tablets Composite analogous to the least marked dosage was taken into beaker of 100 ml. Water (dose not containing free Co_2 added for make up volume to 40 ml. Also mixed on Heavy-Duty Magnetic Stirrer at 300±30 r.p.m. for about 1 minute. Afterward on magnetic stirrer at 300±30 r.p.m. Addition of 10 ml. 0.5 N HCl into the acid sample was done when stirring. After 10 min with continuous stirring pH was recorded.[12]

Acid Neutralizing Capacity (ANC)

By the PAT the extracts had above 3.5 pH. The 250 mg of Extract weighed and added into it some water with one-two drop of polysorbate-80 for stabilization and exactly 5 mlsuspension was made. Then make up volume upto 70 ml with carbonated water among with one minute stirring. Pipetted accurately the 30 ml of 1.0 N Hydrochloric acid and added in suspension while continued stirring upto15 minutes also added one-two drop of phenolphthalein solution. The overconsumed HCl titrated with 0.5 N NaOH (VS) to achieve a steady 3.5 pH. This whole process carried on37±3°Calsodid itagain with taking Gelusil tablet as control sample. The amount of acid taken per gram of antacid were determined in mill equivalents (mEq). [13]

Formula for acid neutralizing capacity (ANC)

Total mEq = (V_{HCl} x N_{HCl}) – (V_{NaOH} x N_{NaOH})

Whereas N_{HCl} and N_{NaOH} are the normality's of HCl and NaOH, respectively, and V_{NaOH} and

$V_{HCl}$is the volume of NaOHand HCl used.

Buffer Capacity (BC)

A precise amount of 5 ml of samples was taken And 50 ml of carbon dioxide free water added in it and kept to 37± 3 °C. The Solution was agitatedcontinuously for 1-2 minutes. By using digital pH meter the initial pHwas noticed.100 mL of 0.1 N HCl was added while constantly stirring. The changes of pH of the solution was monitored with a 5 minutesinterval upto 10 times. The 20 ml of solution was substituted by fresh 20 ml 0.1N HCl by using pipette. This technique was redid every set of 5 minutes until the pH dropped below 2.75 was recorded.[14]

Phagocytosis by C. albican assay process

C. albicans culture was added to the Sabouraud broth & incubated overnight. After overnight culture was centrifuged to get the pellet & supernatant were removed. For 3-4 times collected cell pellet were washed with Hank's balanced salt solution. Obtained Final cell pellet was mixed with blend of Hanks' Balanced Salt Solution (HBSS) & human serum (4:1).By using pricking needle blood was added onto clean glass slide and allowed to incubate at a specific temperature 37°C for 25 min. For remove the clot normal sterile saline was added onto glass slide. Control was prepared for comparison. For the test slides 100ul of different concentration (5mg/ml & 10mg/ml) of test plant extracts were added onto slide and kept for incubation at 37°C for 15 min. Then of predetermined concentration of C. albicans suspension was added & at 37°C for 1 Hr incubation was carried out. The slides were drained, fixed, stained.[15]

Phagocytosis index and percent immune stimulation

The amount of bacteria ingested per phagocyte over a specified duration of incubation of a suspension of bacteria and phagocytes in serum is used to estimate phagocytic activitywas taken as phagocytosis index (PI).[16]

Percent Immunostimulation has been determined using the following formula -

Immunostimulation (%) = PI (Samples) – PI (Control)/ PI (Control)* 100.

PI of sample: Phagocytic index of test plant extract, PI of Control: phagocytic index without test plant extract.

III. RESULTS AND DISCUSSION

Preliminary antacid test

The Control and Extracts samples was showed the more than pH of 3.5. The all samples had showing antacid activity was confirmed. In the comparison the ethanolic extract showed the slightly low and aqueous extract showing greater pH as compared to control sample. All samples considered as antacids because of successfully gone through PAT test. Examined result mentioned in table no.1.

Table 1: PAT of Control and Extracts

Sample	Density	pH On 0.5 N HCl	
		0 min	10 min
Control	1.07	8.15	4.22 ±0.10
Ethyl Acetate Extract	0.96	7.85	3.90 ± 0.07
Aq. Extract	1.11	8.20	6.36 ± 0.18
Ethanolic Extract	1.02	7.98	4.00 ± 0.05

Acid Neutralizing Capacity

$Al(OH)_3$ which is most usable antacid and $Mg(OH)_2$which is ideal antacid along with simethicone (50mg) Which act as defoaming agent used in work as a control sample(Gelusil Tablet).The ANC for the samples were calculated and presented in mill equivalents (mEq) of the antacid, as per theUSP29-NF24 standard.[17]The ANCvalue of ethanolic extract (27.86 ± 10.00)showed the higher value and aqueous extract (24.23 ±23.01)showed lower value. All samples showing ANC above the 5 m Eq hence all samples cleared the requirement according to the united State Food and Drug administration. Recorded results mentioned in table no.2.

Table 2: ANC of Control and Extracts

Sample	pH (on 1.0 N HCl		Volume of NaOH Consumed	mEq of Acid Consumed	ANC mEq/gm of Antacid
	0 min	15min			
Control	8.90	2.73	6.5	26.75	25.00 ± 10
Ethyl Acetate Extract	8.00	2.65	6.5	26.75	27.86±76
Aq. Extract	8.45	3.01	6.2	26.90	24.23 ± 00
Ethanolic Extract	8.37	2.85	6.3	26.85	26.32 ± 13

Buffer capacity

The changes in pH along with duration showing buffering capacity was observed and presented in table no. The pH values lied between 8.51 to the 2.28. The aqueous extract taking much more time (40 minutes) to showing less pH and ethanolic extract maintained pH about 30 minutes. A presentation of a greater ANC as well as a relatively long buffering capacity via a antacid signifies its effectiveness and performance.

Table 3 : Buffering Capacity of Standard and Extracts

Sample	0 min	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min
Control	8.51	3.50	3.91	4.22	3.86	3.27	2.60	ND	ND
Ethanolic Extract	7.91	3.10	3.27	4.00	3.33	3.00	2.72	ND	ND
Aq. Extract	8.45	4.25	4.10	3.75	3.89	3.77	3.23	3.78	2.58

ND- Not Determined

PI and percent Immunostimulation

The phagocytosis index was calculated for further calculation of % immunostimulation. The immunostimulationlied in range 14.18 to 74.28. The ethanolic extract showing the highly phagocytosis index and immunostimulation.

Table 4: PI and % of Immunostimulation

Sample	Phagocytic index		Immuno Stimulation (%)	
	5 mg/ml	10 mg/ml	5 mg/ml	10 mg/ml
Control	70	70	-	-
Ethanolic Extract	35	52	50	74.28
Aq. Extract	10	20	14.28	28.57

The fruit of Citrullus colocynthis (L.) Schard containing 5 mg/ml concentration in aqueous phase shows 14.28% stimulation & at concentration of 10 mg/ml shows 28.57% stimulation. Similarly in ethanolic phase it shows 50 % & 74.28% stimulation respectively

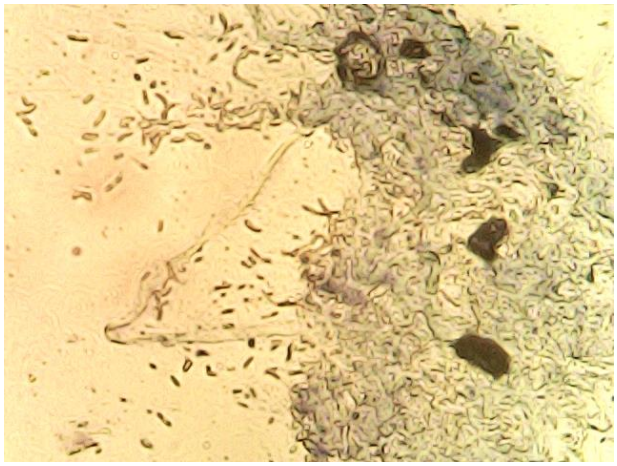

Figure 1: *Candida Albican* cell phagocytized by neutrophils.

IV. CONCLUSION

The analytical study determined theethanolic extract and aqueous extract of fruit of Citrullus colocynthslichard having the higher pH than 3.5 and showed thehaving antacid activity. The more than 5mEq values of acid neutralization capacitywas recorded.The higher ANC demonstrated by the ethanolic extract and theconsistent buffering capacity shown by aqueous extract with comparative to ideal antacids (aluminum hydroxide and magnesium hydroxide with simethicone)to ensure in vitro effectiveness and safety. Along with this the research was done to see if the all sample extract was immune stimulant or immunosuppressant by the phagocytosis of *C. Albican*assay. TheExtracts showed the moderate immune stimulant activity.

V. REFERENCES

[1]. Borhade, P., Deshmukh, T., Patil, V., Kandelwal, K. (2013).Review on Citrullus colocynthis. International Journal of research in Pharmacy and Chemistry, 3(1), 46-53.

[2]. Liu, Y., Chen, G., Chen, X., Chen, S. X., Gan, L. S., & Yuan, T. (2018). Colocynthenins A-D, Ring-A seco-Cucurbitane Triterpenoids from the Fruits of Citrullus colocynthis. Journal of natural products, 81(9), 2115–2119

[3]. Al-Snafi, P. (2016). Medicinal plants with antioxidant and free radical scavenging effects (part2): plant based review. IOSR Journal of Pharmacy (IOSRPHR), 06(07), 62-82.

[4]. Hartwell, J. L., & Abbott, B. J. (1970). Antineoplastic Principles in Plants: Recent Developments in the Field. Advances in Pharmacology, 7, 117–209.

[5]. Jeon, J. H., & Lee, H. S. (2014). Biofunctional constituent isolated from Citrullus colocynthis fruits and structure-activity relationships of its analogues show acaricidal and insecticidal efficacy. Journal of agricultural and food chemistry, 62(34), 8663–8667.

[6]. Arena, J. M. and Drew, R. H. (1986). Poisoning: Toxicology, Symptoms,Treatments, 5th Edn. Charles C. Thomas, Springfield, IL7.

[7]. Memon, U., Brohi, A. H., Ahmed, S. W., Azhar, I., & Bano, H. (2003). Antibacterial screening of Citrullus colocynthis. Pakistan journal of pharmaceutical sciences, 16(1), 1–6.

[8]. Ostovan, F., Gol, A., & Javadi, A. (2017). Investigating the effects of Citrullus colocynthis pulp on oxidative stress in testes and epididymis in streptozotocin-induced diabetic male rats. International journal of reproductive biomedicine, 15(1), 41–48.

[9]. Dixon M. F. (1991). Helicobacter pylori and peptic ulceration: histopathological aspects. Journal of gastroenterology and hepatology, 6(2), 125–130.

[10]. Salisbury B. H. (2021) Terrell J.M. Antacids.[Updated 2021 Jul 7]. In: StatPearls [Internet].Treasure Island (FL): StatPearls Publishing.

[11]. Avorn J. (2011). Learning about the safety of drugs--a half-century of evolution. The New England journal of medicine, 365(23), 2151–2153.

[12]. Code of Federal Regulations, Title 21 - Food and Drugs, 1996, Volume: 5, Section 331.25. Available at: https://www.govinfo.gov/content/pkg/CFR-1996-title21-vol5/xml/CFR-1996-title21-vol5-sec331-25.xml

[13]. United State Pharmacopoeia (USP) GC05 USP29-NF24, Page-2561.Available at: http://www.pharmacopeia.cn/v29240/usp29nf24s0_c301.html

[14]. Joshi. D., Jain, Y., Malviya, S., Kharia, A. (2017). Preparation and biological standardization of antacid formulation. World Journal of Pharmaceutical Research, 6(15), 716-721.

[15]. Ponkshe, C. A., & Indap, M. M. (2002). In vivo and in vitro evaluation for immunomodulatory activity of three marine animal extracts with reference to phagocytosis. Indian journal of experimental biology, 40(12), 1399–1402.
[16]. Definition of Phagocytic index. Medical Dictionary, Merriam-Webster, Merriam-Webster.com. Available at:https://www.merriam-webster.com/medical/phagocytic%20index
[17]. US FDA. (1774). Rules and regulations, Fed Regist. 39 (108)19874.

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