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Original Research Article

Anti-inflammatory role of scent leaf (*Ocimum Gratissimum L.*) and bitter leaf (*Vernonia Amygdalina D.)* on simulated gastrointestinal digestion

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Abstract

Background: Inflammation can be described as a part of the body's immune response to any kind of injury or infection. Some of its symptoms include pain, redness, heat and swellings. Inflammation could be acute or chronic depending on the type. Many drugs have been developed to tackle different form and kinds of inflammations. Most of these drugs are called non-steroidal anti-inflammatory drugs [NSAID]. Many of these drugs have side effects. Therefore, most researchers focus their research activities and objectives in exploring less toxic and yet effective drugs that can serve as an effective anti-inflammatory drugs with little or no side effects. Numerous research have shown that plants have vast medicinal properties which are well documented in several publications. The anti-inflammatory potential of medicinal plants stands out amongst its numeorus medicinal values and have been employed in ethno- medicine and modern medicines.
Aim and Objectives: This research is aimed at investigating Anti-inflammatory role of scent leaf (*Ocimum Gratissimum* L.) and bitter leaf (*Vernonia Amygdalina* D.) on simulated gastrointestinal digestion.
Materials and Methods: Methodologies employed here are well elucidated in this article.
Results: The results obtained showed a remarkable decrease in all inflammatory parameters
Conclusion: The research justifies and makes these plants (*Ocimum Gratissimum* L.) and bitter leaf (*Vernonia Amygdalina* D.) a potential anti-inflammatory drugs.

Keywords: Inflammatory, Sent Leaf, Bitter leaf, Anti-inflammatory drugs, Medicinal plant.

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1. Introduction

Vernonia amygdalina (VA) is commonly called bitter leaf, mostly due to its bitter taste. It is a common shrub or small tree grown in tropical Africa. It is also well distributed in Asia and are commonly found along drainage lines and in natural forest or commercial plantation. Vernonia amygdalina (VA) belongs to the Asteraceae family and called different names in many African ethnic groups some of the names are; 'African bitter leaf' in Africa, 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Ilo' in Igala, 'Oriwo' in Edo, 'Chusar-doki' in Hausa, 'Grawa' in Amharic and 'Omubirizi' in south- western Uganda. The leaves are green in colour with a characteristic odour and bitter taste.[1] In particular, it is used as a spice in the Cameroonian dish "Ndole" and to make the well-known Nigerian bitter leaf soup, "Onugbo".[2] Because of its many therapeutic qualities, Vernonia amygdalina (VA) is utilized in the majority of African nations to cure conditions like malaria, infertility, diabetes, gastrointestinal issues, and STDs[3] Their traditional and ethnomedical applications are not just for people; they are also advantageous for animals as a supplement to animal feed. In Northern Nigeria, it is commonly given to horse feed as "Chusan Dokin," a tonic that strengthens or fattens.[4]

However, Ocimum gratissimum, sometimes referred to as smell leaf, is a fully grown flowering plant that has systems

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for roots, stems, and leaves.[6] The essential oil found in the leaves and stems of this plant is the reason it is grown. A significant insect repellant is these essential oils. In addition, the essential oil and the entire plant have numerous uses in traditional medicine, particularly in Indian and African ethnomedicine. This plant's therapeutic applications are being investigated for the treatment of a variety of illnesses, including pneumonia, ocular, skin, fever, diarrhea, and headaches.[7,5] This plant's seeds are used to cure gonorrhea traditionally because they have laxative qualities. The essential oil is used to cure skin conditions, diarrhea, stomach ache, fever, and inflammations of the throat, ears, or eyes. It is undergoing antimicrobial testing. Whereas the leaves are used as a flavoring in Thailand, they are used to make tea in Indonesia (Sumatra). In addition to its many therapeutic uses, the eugenol-type of O. gratissimum is planted in cemeteries and used in ceremonial body washing in various nations, such as Indonesia. Additionally, O. gratissimum, sometimes known as "ram tulsi," is frequently used in religious rites and ceremonies in India.[8]

An evolutionarily conserved process of defense and a vital survival mechanism,[12] inflammation is a series of intricate changes in the tissue that aid in removing the original cause of the cell injury, which may have been brought on by physical agents (burns, radiation, and trauma), chemicals (caustic substances), or infectious agents or substances from their metabolism (microorganisms and toxins).[13,14] Localized redness, swelling, discomfort, heat, and loss of function are the clear indicators of inflammation. These symptoms are caused by immunological, physiological, and metabolic alterations that increase blood flow, vascular permeability, and leukocyte recruitment by releasing pro-inflammatory chemical mediators at the site of injury.[11]

An inflammatory response involves macrophages, neutrophils known to secrete different mediators that are responsible for the initiation, progression, persistence, regulation, and eventual resolution of the acute state of inflammation. The resolution of inflammation is influenced by several anti-inflammatory mediators and the recruitment of monocytes for the removal of cell or tissue debris. Basically, inflammation could be acute or chronic and this is dependent on the ability of anti-inflammatory mediator in conjunction with monocyte to remove cell or tissue debris on time by normal cellular process. The acute phase may develop into a chronic phase, and in both industrialized and developing nations especially in Africa the burdens associated with pathological illnesses are significantly influenced by this chronic inflammation. For example, obesity-related diabetes due to insulin resistance is known to be influenced by chronic inflammation.[9,10] Therefore, it is necessary to search for additional powerful natural anti-inflammatory medications, most especially from herbal medicines with low toxicity and easy accessibility.[15] This was the rationale for the design of this study.

2. Materials and Methods

Plant material collections we bought fresh mature leaves of Vernonia amygdalina (bitter leaf) and Ocimum gratissimum (scent leaf) from "oja oba," a local market in Akure, Ondo state, Nigeria. Voucher specimens were placed at the Herbarium after identification and authentication were completed at the Federal University of Technology, Akure (FUTA) Department of Crop, Soil, and Pest Management**.**

2.1. Methods

2.1.1. Preparation of plant extracts

After giving the fresh, developed leaves of Vernonia amygdalina (bitter leaf) and Ocimum gratissimum (scent leaf) a thorough water wash, the edible and inedible parts were separated. After being cleaned and cut into smaller pieces, the edible parts were allowed to air dry for two weeks at room temperature (240C). An electric blender was used to grind the dried leaves into a fine powder.

2.2. Extraction

2.2.1. Methanol extraction

By soaking 10g of the powdered plant in 200 mL of 100% methanol, the methanol extracts of both plants were made. For eighteen hours, the combination was incubated at 40°C in a shaking water bath. The mixture was then filtered every six hours, and the filtrate residues were reconstituted into another 200 milliliters of methanol, a process that was done three times. We therefore used Whatmann filter paper No. 1 (125 mm) to filter the final extracts.

To achieve a dark green semi-solid, the filtrate was vacuum-concentrated using a rotary evaporator (ideally, the temperature should be between 40°C and 45°C to prevent denaturation of the active components). A sterile bottle was filled with the filtrate. The extract was kept in a refrigerator for later use after being diluted fifty times.

2.3. Experimental design

We used adult male Wistar rats weighing 150–170 g. At the Federal University of Technology, Akure's Department of Biochemistry Animal House, the rodents were housed in standard conditions and fed standard rodent chow (Vita Feeds Nigeria Limited) along with unlimited water. Every experiment was carried out in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals' standard operating procedures. The rats were divided into four groups, each consisting of eight rats.

1. **Group 1:** Digested *Vernonia amygdalina* (bitter leaf)
2. **Group 2:** Digested *Ocimum gratissimum* (scent leaf)
3. **Group 3:**Undigested *Vernonia amygdalina*
4. **Group 4:** Undigested *Ocimum gratissimum*

Slightly modified approach was used to model gastrointestinal digestion.[16] To assess the in vitro

bioavailability of the antioxidant qualities of Vernonia amygdalina (bitter leaf) and Ocimum gratissimum (scent leaf), the procedure involved imitating the conditions of the stomach and small intestine.

2.4. Procedure

For an in vitro stomach digesting procedure, 40 mL samples (i.e., 2g of powdered samples in 40 mL of distilled water) were incubated in a shaker water bath (200 rpm) at 370 C for 10 minutes. The mixture was then incubated in a shaker water bath at 37^0 C for 10 minutes after 300 µL of α-amylase was added to stimulate "the partial digestion in the mouth which breaks down polymeric starch into a shorter oligomers, but upon reaching the gut, this partially digested starch will extensively hydrolyze into a smaller oligosaccharides by the α-amylase synthesized in the pancreas".[17] Thus, the pH of the extracts was then adjusted to 2.0 with 1M HCl solution which stimulate the acidic environments of the stomach; these were transferred back again to a shaker water bath at 37^0 C for 30 min.

After adding swine gastric mucosa pepsin (15.750 units EC 3.4.23.1) to the acidified extract, the mixture was incubated for 20 minutes at 37^0 C in a shaker water bath (2000 rpm). Following incubation, the samples were neutralized with NaOH to bring the pH down to 6.0. The mixture was then incubated once more for 20 minutes at 37^0 C in a shaker water bath. The resulting mixture was then incubated at 37^0 C for an additional 20 minutes after 10 ml of pancreatin from pig pancreas (8 × USP requirements) and bile salts (10 mM) solution were added. Lastly, 1M NaOH was used to bring the solution's pH down to 7.5 once more. After an hour of centrifugation, a 20-fold aliquot of the stomach digested samples was taken from the supernatant and stored in a refrigerator for later use. Three duplicates of the in vitro digestion process simulation were run.

3. Harvesting of Organ and Homogenization

After rendering the adult albino rats unconscious, the liver and brain tissue—the liver and the complete brain, respectively—were quickly separated, put on ice, and weighed. The tissues were then homogenized in a Teflon-glass homogenizer using around ten up-and-down strokes at a speed of 1,200 rev/minute in cold Tris HCl buffer (pH 7.4) (1:10 w/v). The low speed supernatant (S1) was retained for the lipid peroxidation test after the homogenate was centrifuged for 10 minutes at 3000g to produce a pellet that was disposed of.[18]

4. Anti-Inflammatory Activity

4.1. Membrane stabilization

4.1.1. Preparation of Red Blood Cells (RBCs) suspension

A healthy Wistar rat that had been acclimatized was used to obtain the blood. The centrifuge tubes were filled with the extracted blood. For ten minutes, the tubes were centrifuged at 3000 rpm. According to,19 [19], the volume of blood was measured and reconstituted using a 10% v/v solution with regular saline. This process is known as heat-induced hemolysis. One milliliter of test sample at varying concentrations (100–500 µg/mL) and one milliliter of 10% RBC suspension made up the reaction mixture (2 mL); in the control test tube, saline was used in place of the test sample. Diclofenac was a common medication. Every centrifuge tube holding the reaction mixture was incubated for 30 minutes at 560 C in a water bath. The tubes were cooled with flowing tap water at the conclusion of the incubation period. The absorbance of the supernatants was measured at 560 nm after the reaction mixture was centrifuged for five minutes at 2500 rpm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \text{ X } 100$$

$$\% \text{ Inhibition} = \frac{\text{Percent membrane stabilization was calculated by the method of Shinde } et\ al. 1999;\ \text{Saket } et\ al. (2010).}{100 - (A1 - A2) \quad \text{X } 100\ A0}$$

Where; A1 is the absorbance of the sample,
A2 is the absorbance of the product control and,
A0 is the absorbance of the positive control

4.1.2. Antiproteinase action

The test was conducted using Sakat et al. (2010)'s modified methodology. 0.06 mg of trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4), and 1 ml of test sample with varying quantities (100–500 µg/ml) were all present in the reaction mixture (2 mL). After five minutes of incubation at 37^0 C, 1 mL (already mentioned so use the term ml) of 0.8% (w/v) casein was added to the mixture. Another twenty minutes were spent incubating the combination. To stop the process, 2 mL of 70% perchloric acid were added. After centrifugation of the hazy suspension, the absorbance of the supernatant was measured at 210 nm using buffer as a blank. The experiment was performed in triplicate, and the proportion of proteinase inhibitory activity that was inhibited was determined.

4.2. Statistical analysis

Every analysis was carried out three times. Microsoft Excel software (Microsoft Corporation, Redmond, WA) was used to calculate the results initially. Mean ± SD was used to express the results. Using the program Graph Pad Instat, the One-Way Analysis of Variance (ANOVA) and Dunnet Multiple Comparison test were used to compare the differences between the experimental groups. A significance level of $p<0.0001$ was established.

5. Results

Figure 1: Anti-inflammatory effects of heat-induced hemolysis (mg/ml) of the methanol extract and digested enzymes of Vernonia amygdalina and Ocimum gratissimum (mg Diclofenac equivalent/g of the sample).

As concentration increased, the extracts' scavenging capability gradually increased as well. Mean ± SD of separate experiments conducted in triplicate was used to express values. According to the Tukey Test, bars with signs are significantly different (P<0.0001).

DVA = Digested *Vernonia amygdalina* (Bitter Leaf); DOG= Digested *Ocimum gratissimum* (Scent Leaf); UVA= Undigested *Vernonia amygdalina*; UOG= Undigested *Ocimum gratissimum* *** represents stastistical difference at p<0.0001 between the standard and all the extracts at all concentration, and between DVA and UVA, DOG and UOG at varing concentration; *** represents stastistical difference at p<0.0001 between DVA and DOG(at 10 and 50mg/ml); * represents stastistical difference at p<0.0001 between DVA and DOG at 40mg/ml while while ns represent no significantly difference at (p< 0.0001) between DOG and UVA (10mg/ml), DVA and DOG(30mg/m) and, UOG and DVA (at 40 and 50mg/ml).

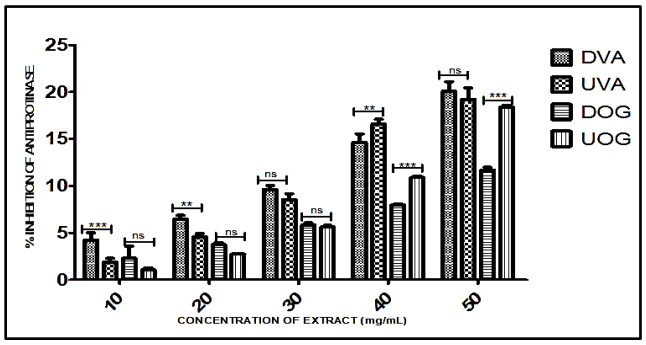

Figure 1: Anti-inflammatory activity of the methanol extract and enzymes digest of *Vernonia amygdalina* and *Ocimum gratissimum* on % inhibitory activity of proteinase (mg trypsin equivalent/g of the sample).

A gradual increase in scavenging potential of the extracts was obtained with an increase in concentration. Values are expressed in Mean ± SD of independent experiments performed in triplicate. Bars with signs are significantly different (*P*< 0.0001) by Tukey Test.

DVA = Digested *Vernonia amygdalina* (Bitter Leaf); DOG= Digested *Ocimum gratissimum* (Scent Leaf); UVA= Undigested *Vernonia amygdalina*; UOG= Undigested *Ocimum gratissimum*

**** Represents statistical difference at p<0.0001 between DVA and DOG; DVA and UVA; DVA and UOG; DOG and UVA; DOG and UOG at all concentration; *** represents statistical difference at p<0.0001 between UVA and DVA at 10mg/ml; ** represents statistical difference at p<0.0001 between UVA and DVA, DVA and DOG while ns represent no significant difference at (p< 0.0001) between DVA to UOG.

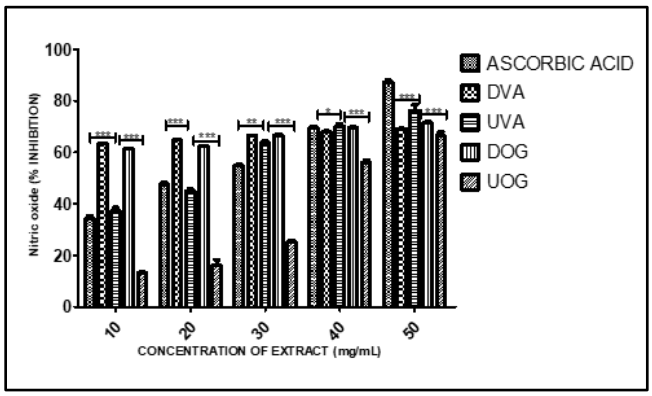

Figure 2: Anti-inflammatory activity of the methanol extract and enzymes digest of *Vernonia amygdalina* and *Ocimum gratissimum* on % inhibitory activity of Nitric Oxide (mg Ascorbic acid equivalent/g of the sample).

A gradual increase in scavenging potential of the extracts was obtained with an increase in concentration. Values are expressed in Mean ± SD of independent experiments performed in triplicate. Bars with signs are significantly different (*P*< 0.0001) by Tukey Test.

DVA = Digested *Vernonia amygdalina* (Bitter Leaf); DOG= Digested *Ocimum gratissimum* (Scent Leaf); UVA= Undigested *Vernonia amygdalina*; UOG= Undigested *Ocimum gratissimum*

**** Represents statistical difference at p<0.0001 between the standard to all the concentration at 50mg/ml, DOG and UOG, DOG and UVA at 10 and 40mg/ml, while ns represent no significant difference at (p< 0.0001) between DVA and DOG at all concentration and, between standard to UVA at 40mg/ml.

6. Discussion

Over the years, scientists have investigated plants for a variety of uses, including their nutritional and therapeutic qualities. As a result, many scientific studies now focus on plants. It's interesting to note that a plant's secondary metabolites, which safeguard the plant against disease and pests while simultaneously having important effects on human health, are linked to the plant's therapeutic secret. Bitter leaf and scent leaf are two of the plants that have been investigated, and numerous medical benefits have been verified. This study was designed to assess the role of these plants in regulating inflammation because little is known about their anti-inflammatory qualities. The study's findings are divided into two categories.

6.1. Heat induced hemolysis

The results of heat-induced hemolysis showed that the methanol extract of Ocimum gratissimum and the enzyme digest of Vernonia amygdalina both exhibited a strong degree

of heat-induced inhibitory action. The standard is supposed to rise with concentration, and the results also showed that the methanol extract of Vernonia amygdalina exhibited the highest degree of heat-induced inhibitory action.

6.2. Anti-proteinase

The enzyme digest of Vernonia amygdalina and the methanol extract of Ocimum gratissimum demonstrated a competitive level of % inhibitory activity of proteinase, according to the sample's anti-proteinase activity, as displayed in **Figure 2**. The results also showed that, at all concentrations, the enzyme digest of Vernonia amygdalina leaf extract has the maximum potency when compared to Ocimum gratissimum.

6.3. Nitric oxide

Nitric oxide content, which is given as mg ascorbic acid equivalent/g of the sample (mgAAE/g), that is the antioxidant activity of the sample is compared to a standard curve made with ascorbic acid (Vitamin C), and it tells you how much antioxidant capacity the sample has, expressed as if it were Vitamin C. The findings demonstrated that the study plants' undigested and digested samples successfully suppressed nitric oxide.

7. Conclusion

Based on the aforementioned results, it can be said that Ocimum gratissimum (scent leaf) and Vernonia amygdalina (bitter leaf) have some antioxidant and anti-inflammatory qualities.

8. Source of Funding

None.

9. Conflict of Interest

None.

References

1. Akpaso MI, Atangwho IJ, Akpantah A, Fischer VA, Igiri A, Ebong E. Effect of Combined Leaf Extracts of Vernonia amygdalina (Bitter Leaf) and Gongronema latifolium (Utazi) on the Pancreatic β-Cells of Streptozotocin. *Brit J Med & Medical Rese*. 2011;1(1):24-34. https://doi:10.9734/BJMMR/2011/215
2. Ho WY, Liang WS, Yeap SK, Beh BK, Yousr AHN. In vitro and in vivo antioxidant activity of Vernonia amygdalina water extracts, *Afric J Biotech*., 2012;11(17):4090–4. https://doi:10.5897/AJB10.1639
3. Farombi EO, Owoeye O. Antioxidant and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *Int J of Environ Res Pub Health.* 2011;8:2533–55. https://doi:10.3390/ijerph8062533
4. Hamzah RU, Jigam AA, Makun HA, Egwin EC. Antioxidant Properties of Selected African Vegetables, Fruits and Mushrooms: A Review. *Intech* 2013;9:203-50.
5. Ilori, M.,Sheteolu, A.O., Omonibgelin, E.A. and Adeneye, A.A. (1996). Antibacterial activity of Ocimum gratissimum (Laminaceae). s. 14: 283-5. https://doi:10.1590/S0074-02761999000500022
6. Iwu MM. Handbook of African Medicinal plants. CRC Press, New York. 1993;Pp: 214-215.
7. Onajobi FD. Smooth Muscle Contracting Lipid Soluble Principles in Chromatographic Fractions of Ocimum gratissimum. *J Ethanopharm*. 1986;18(1):3-11. doi:10.1016/0378-8741(86)90038-3
8. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009). Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. http://www.worldagroforestory.org/site/treedatabases.asp
9. Ahmed A.U. An overview of inflammation: mechanism and consequences. *Front Biol.* 2011l;6(4):274–81. https://doi:10.1007/s11515-011-1123-9
10. Hotamisligil G.S. Inflammation and metabolic disorders. *Nature.* 2005;;444(7121):860–7. https://doi:10.1038/nature05485
11. Virshette SJ, Patil MK, Somkuwar AP. A review on medicinal plants used as anti inflammatory agents. *J Pharmacogn Phytochem*. 2019;8(4):1641–6.
12. Liu CH, Abrams N, Carrick DM, Chander P, Dwyer J, Hamlet MRJ. Biomarkers of chronic inflammation in disease development and prevention: Challenges and opportunities. *Nat Immunol*. 2017;18(11):1175–80. https://doi: 10.1038/ni.3828.
13. Fialho L, Cunha-E-Silva JA, Santa-Maria AF, Madureira FA, Iglesias AC. Comparative study of systemic early postoperative inflammatory response among elderly and non-elderly patients undergoing laparoscopic cholecystectomy. *Rev Col Bras Cir.* 2018;45:e1586. https://doi:10.1590/0100-6991e-20181586.
14. Jang CH, Kim YY, Seong JY, Kang SH, Jung EK, Sung CM, Kim SB, Cho YB, Sung JY. Clinical characteristics of pediatric external auditory canal cholesteatoma. *Int J Pediatr Otorhinolaryngol.* 2016;87:5–10. doi: 10.1016/j.ijporl.2016.05.029.
15. Esther U. U., Emmanuel O. I., Olaide B. B., Adewale A. O., Godspower O. (2025). "Therapeutic roles of Aframomum Melegueta on Testosterone Propionate Induced Benign Prostrate Hyperplasia in Wistar Rats. *IJCBR* 12(1):24-30. https://doi:10.18231/j.ijcbr.2025.004.
16. Gil-Izquierdo A., Gil MI, Ferreres F. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J Agricultural Food Chem*. 2002;50(18):5107-14.
17. Truscheit E, Frommer W, Junge B, Müller L, Schmidt DD, Wingender W. Chemistry and biochemistry of microbial α-glucosidase inhibitors. Angewandte Chemie International Edition in English, 1981;20(9):744-61. https://doi.org/10.1002/anie.198107441
18. Di Bella G, Mascia F, Gualano L, Di Bella L. Melatonin anticancer effects. *Int J Mol Sci*.2013;14(2):2410-30. https://doi:10.3390/ijms14022410
19. Kulkarni A, Govindappa M, Chandrappa CP, Ramachandra YL, Koka PS. Phytochemical analysis of Cassia fistula and its in vitro antimicrobial, antioxidant and anti-inflammatory activities. *Adv Med Plant Res.* 2015;3(1):8-17.

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