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Screening of Traditional wild Edible Fruits for Presence of Lectins with Immunomodulatory Efficacy

B.M. Siddesh[1], B.K. Kiran[1], Ankith Sherapura[1], Banumathi[1], Riaz Mahmood[2] and B.T. Prabhakar[1*]
[1]Molecular Biomedicine Laboratory, Postgraduate Department of Studies and Research in Biotechnology, Sahyadri Science College, Kuvempu University, Shivamogga (Karnataka), India.
[2]Postgraduate, Department of Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta (Karnataka), India.

(Corresponding author: B.T. Prabhakar)*
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ABSTRACT: Wild edible fruits (WEF's) are recognized for their nutritional and medicinal properties, particularly due to their content of lectins, which are carbohydrate binding proteins that exhibit various biological activities. Many fruits contain lectins and exhibit various biological properties including immunomodulation, cytotoxicity and mitogenic activity and being used as therapeutic agents against cancer. In present investigation, eight Wild Edible Fruits (WEFs) which are uncultivated, but widely consumed on different season, *viz Cordia myxa* (CM), *Cucumis melo* var. *Cantalupensis (CMC), Cucumis melo var momordica* (CMM), *Malpighia emarginata* (MAE), *Mimusops elengi (ME), Ziziphus mauritiana* (ZM), *Mintingia calabura* (MC) and *Limonia acidissima* (LA) were screen for presence of immunostimulant lectin. Among them, partially purified fruit extracts of CM, *CMC,* CMM, MAE, *ME and* ZM exhibited Hemagglutination activity confirming the presence of lectin. The selected plants further screened for presence of immunomodulatory cytokines by ELISA, Results inferred that, elevated levels of IL-2, IL-10, IL-12 were noticed. Immunomodulation studies by treating conditioned media obtained from selected fruit CM against multiple cancer cell lines inferred that, it induces significant expression of immune stimulant cytokines and its antiproliferative response against human lung adenocarcinoma (A549) and murine ascites cell line (EAC). Overall, the study postulates the importance of the six WEFs for the first time with immunomodulatory potentiality and highlights the importance of WEF's with therapeutic potentiality.

Keywords: Wild edible fruits, *Cordia myxa*, Lectins, Immunomodulation and Antiproliferation.

INTRODUCTION

Plants are abundant source of natural compounds that have been used for medicinal purposes by various cultures and societies for centuries. Plant based herbal medicines have been used to treat various diseases since ancient times and have been utilized to enhance human health since the dawn of medicine (Aye *et al.,* 2019). Natural sources and herbal medicines are attracting attention because they are more effective, safe, culturally acceptable, and have fewer negative effects than manufactured pharmaceuticals. Especially, fruits are rich source of proteins, minerals, vitamins, fats, carbohydrates and other essential phytochemicals that have anti-malnutrition properties and tackle a variety of health problems like inflammation, obesity, diabetes, cardiovascular disease, and cancer (Singh *et al.,* 2014).

Wild edible fruits (WEF's) are an often-overlooked category of food that can significantly contribute to nutrition and health. These fruits, which grow in natural habitats without cultivation, are rich in essential nutrients, vitamins and bioactive compounds. They play a crucial role in food security, particularly in rural communities where access to conventional agricultural products may be limited (Li *et al.,* 2016). WEFs are beneficial not only for their nutritional content, but also for their medicinal attributes. WEF provide several health advantages. They are known to contain a large number of antioxidants, vitamins and other bioactive compounds which exhibit numerous pharmacological activities (Suvarna Gaikwad *et al.,* 2023; Duguma, 2010). Also these fruits reportedly contain various lectins that contribute to their nutritional and medicinal properties, including immune modulation, antimicrobial activity and anticancer effects, enhancing the health benefits associated with consuming these fruits (Souza *et al.,* 2013).

Recently wild edible fruits are gaining much importance in scientific research due to their multiple pharmacological attributes. For instance, *Cordia myxa* extract exhibit significant antioxidant and antibacterial activity (Al-Musawi *et al.*, 2022; Matcheme *et al.,* 2023). Another study reported, synthesis of zinc oxide nanoparticles from *Cordia myxa e*xhibit cytotoxicity against human colon cancer (HCT116) (Nagaraja *et al.,*

2023). *Cucumis melo var momordica* extract demonstrated effective antioxidant, antidiabetic and anti-anxiety effects in animal models (Yadav *et al.,* 2022; Tiwari, 2023). *Malpighia emerginata* extract exhibited potent antioxidant and antimicrobial activity (Malaguti *et al.,* 2023; October *et al.,* 2024). *Mimusops elengi* extract demonstrated apoptotic and antiproliferative efficacy in Ehrlich ascites carcinoma cells (Kar *et al.,* 2022). *Ziziphus mauritiana* extract shows significant antioxidant and wound healing activity (Shady *et al.,* 2022). *Muntingia calaburo* fruit extract demonstrated antioxidant and antimicrobial activity (Ariffin *et al.,* 2022; Nur *et al.,* 2022) and synthesized silver nanoparticle from *Limonia acidissima* shows significant antioxidant and antimicrobial activity (Daphedar *et al.*, 2024)

Lectins are a unique family of proteins found in nature that can selectively detect and bind to carbohydrates and glycoconjugates without altering the carbohydrate structure. These proteins are most abundant in plants, The lectins are differed in their amino acid sequences, carbohydrate specificity, molecular weight, stability and constituent beauty products. Despite their differences, many lectins have similar of biological processes, including cell interaction and immune system response and also exhibit various pharmacological properties such as anticancer, antiviral, antifungal, antiparasitic, and immunomodulatory actions (Mishra *et al.,* 2019). Plant lectins have attracted a lot of attention in glycobiology and medical research because of their unique function. According to research, lectins may efficiently connect with cell surfaces, enabling crucial physiological functions to happen. One of their primary strengths is to agglutinate cells or precipitate glycoconjugates, making them useful in cell biology and immunology research. Lectins' carbohydrate structure specificity makes it possible to be utilized in therapeutic applications, such as drug delivery systems that target specific tissues or cells. Certain lectins, for example, can bind to particular glycan moieties on cell surfaces, allowing beneficial prescription drugs to be delivered with increased accuracy (Bah *et al*., 2013; Coelho *et al*., 2017). As research continues to explore the full range of benefits offered by WEF and their lectins, they may become increasingly valued for their contributions to health and well-being (Singh *et al*., 2014).

Most of these reported lectins are widely cultivated and consumed. The major focus of the current investigation is to screen the seasonal WEFs which are uncultivated but are having traditionally medicinal importance. Emphasis was given to identify the fruits for presence of lectin having immunomodulation activity. The study employs the isolation, partial purification and biological characterization of lectin and evaluation of immunomodulatory activity and antiproliferative activity of lectins isolated from 8 commonly used WEF's (Table 1) and identification of potential fruit lectin from CM with detailed investigation.

MATERIALS AND METHODS

Cell lines of human origin such as melanoma (A375), lung adenocarcinoma (A549), squamous cell carcinoma (A388), breast adenocarcinoma (MCF-7), cervical cancer (Hela), hepatocellular carcinoma (HEPG2), normal bronchial epithelial cells (BEAS-2B) and mouse origin such as earlich ascites carcinoma (EAC) and fibroblast cells (NIH-3T3) were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cell culture wares were obtained Eppendorf, Germany. Culture media, DMEM and RPMI, Penicillin and Streptomycin solution, FBS were procured from Invitrogen, USA. Ficoll-Hypaque, Trypsin, and 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide (MTT) were obtained from Sigma Aldrich (USA), All other general chemicals were from Hi-media laboratories, India. Photographs were taken using Canon power shot Sx500 IS camera.

Isolation and partial purification of dietary lectins

Selection, Collection of WEF's: The eight uncultivated and widely used edible fruits were collected from different region of Karnataka, India, in different seasons (Table 1). The collected fruits were identified and authenticated by botanist, Dr. Ramesh Babu, Professor, Department of Botany, Sahyadri Science College, Kuvempu University, Karnataka, India. The voucher specimens of collected fruits *Cordia myxa* (No.BT/WEF/CM/01), *Cucumis melo var cantalupensis (*No.BT/WEF/CMC/02**),** *Cucumis melo var momordica (*No.BT/WEF/CMM/03**),** *Malpighia emerginata* (No.BT/WEF/MAE/04), *Mimusops elengi* (No.BT/WEF/ME/05), *Ziziphus mauritiana* (No.BT/WEF/ZM/06), *Muntingia calaburo* (No.BT/WEF/MC/07) *and Limonia acidissima* (No.BT/WEF/LA/08) were maintained in the Molecular Biomedicine Laboratory, Post Graduate Department of Biotechnology, Sahyadri Science College, Kuvempu University, Shivamogga, Karnataka, India.

Preparation of crude extract and partial purification of lectins: Selected WEFs were washed and cleaned with sterile water. The latex extract was obtained by creating several orifices in the epicarp of the CMC and CMM fruit with a sterile needle and the oozed-out latex sap was diluted with 10 mM PBS (pH 7.4) at a 1:1 ratio. The pulp was extracted from the CM, MAE, ME, ZM, MC and LA fruits using sterile scalpels and spatulas and homogenized with 10 mM PBS (pH 7.4) in a 1:10 (10%, v/v) proportion as described before (Vigneshwaran *et al.,* 2016). After homogenization, the suspension of each fruit was centrifuged at 5000 rpm for 5 minutes at 4°C to remove debris. The clear supernatants were collected and purified using ammonium sulphate precipitation and dialysis. The purified WEF's extract was estimated for protein and aliquots were stored for further experiments.

Hemagglutination (HA) assay: Hemagglutination (HA) assay was performed on a 2% trypsinized chick erythrocyte suspension, as previously described. To visualize agglutination, 100 µl of 2% trypsinized erythrocytes were combined with an equivalent amount

of serially diluted protein solution on a concavity agglutination plate. The mixture was stirred gently and incubated at 37°C for 1 hr. To assess the HA inhibition assay, different sugar concentrations were incubated with WEF's for 1 hr at 37°C, and the hemagglutination assay was performed (Vigneshwaran *et al.,* 2017).

Cell proliferation studies

Proliferation index studies: Human peripheral blood lymphocytes (HPBL's), murine splenocytes and thymocytes were isolated as described earlier and cultured in RPMI medium in a humidified environment at 37°C (Clement F *et al.,* 2010). The human lymphocytes, murine splenocytes, thymocytes and Raw 246.7 were cultured in 96 well plates (200 µl of 2.5 × 10^6 cells/ml) in a CO_2 incubator at 37°C with 5% CO_2. After 24 hrs, confluence cells were treated with partially purified extract of WEF's (0 to 25 µg/ml) & reference mitogen Con A (0 to 25 µg/ml) for 48 hrs and MTT assay was carried out to measure the mitogenic activity of lectins.

ELISA: The cytokines IL-2, IL-10, and IL-12 in the condition media treated by WEF's were quantified using ELISA. In brief, 100µL of the condition media were coated on 96-well microtiter ELISA plates using coating buffer and incubated at 4°C. In addition, the wells were rinsed and incubated with the corresponding primary and secondary antibodies, and then re-incubated with the corresponding antibodies. The protein level was quantified by measuring absorbance at 405 nm using a microplate ELISA reader (Raytomicroplate reader) (Clement *et al.,* 2010).

Table 1: List of edible fruits.

Sr. No.	Wild edible fruits	Family	Vernacular name	Part used
1.	*Cordia myxa*	Boraginaceae	Assyrian plum	Pulp
2.	*Cucumis melo* var *cantalupensis*	Cucurbitaceae	Cantaloupe	Sap
3.	*Cucumis melo* var *momordica*	Cucurbitaceae	Snap melon	Sap
4.	*Malpighia emerginata*	Malpighiaceae	Barbados Cherry	Pulp
5.	*Mimusops elengi*	Sapotaceae	Spanish cherry	Pulp
6.	*Ziziphus mauritiana*	Rhamnaceae	Indian jujube	Pulp
7.	*Muntingia calabura*	Muntingiaceae	Jamaica cherry	Pulp
8.	*Limonia acidissima*	Rutaceae	Wood apple	Pulp

Antiproliferative efficacy: The above-mentioned cancer and normal cell lines were cultured and maintained in appropriate medium with 10% FBS in 5% CO_2 at 37°C. For antiproliferative studies, HPBL's were treated with WEF's fruit extracts (0, 10 and 15 µg) conditioned media was diluted 1:1 and treated against various cell lines. After 48 hrs MTT, trypan blue dye exclusion and LDH leak assay was carried (Vigneshwaran V *et al.* 2017).

Statistical Analysis: All experiments were repeated three independent times and statistically analysed through one-way ANOVA followed by Brown–Forsythe test. The results were expressed as mean ± SD. Statistical significance was set at *$p<0.05$ and **$p<0.01$. All data analyses were performed using Graph pad prism version 8.0.

RESULTS

Six WEF's extract proliferates immune cells through immune cytokines: The six chosen WEF's extract have been evaluated for immune cell proliferation with HPBL's, murine splenocytes, thymocytes and Raw 246.7 cells. The results revealed that CM fruit extract initiates stimulation of immune cell proliferation at 10 µg concentration and attains saturation at 15 µg while other WEF's extract shows moderate immune cell proliferation. The proliferative index of WEF's extract was reported as a fold increase relative to the control and denoted as 1.0. The proliferative index of CM extract was 2.09 in HPBL's, 1.52 in splenocytes, 1.9 in thymocytes and 1.78 in Raw 246.7 cells at concentration, while other WEF extracts showed no significant influence on cell growth (Fig. 2 A-D).

Furthermore, the amount of immune cell proliferation was assessed by validating the secreted immune cytokines in HPBLs. The results infer that, IL-2, IL-10 and IL-12 levels were significantly high in CM treated conditioned media among six WEF's treated against HPBL's. The levels of IL-2 was 2.8 & 3.67, IL-10 was 2.04 & 3.03 and IL-12 was 2.03 & 3.01 folds high at 10 and 15 µg/ml concentration respectively. While other WEFs stimulated condition media show moderate to no significant secretion of IL-2, IL-10 and IL-12 (Fig. 2 E-G).

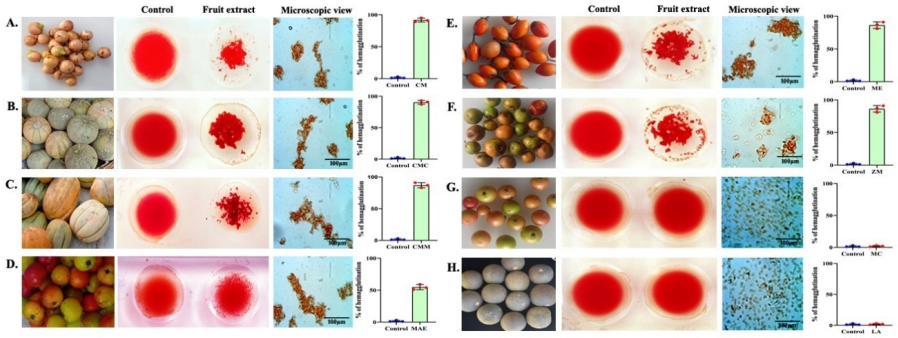

Fig. 1. Six WEF's extract exhibits lectin activity by agglutinating blood cell: The lectin activity of WEF's extract was evaluated by HA assay. (**A**) CM. **(B)** CMC. **(C)** CMM. **(D)** MAE. (**E**) ME. **(F)** ZM. **(G)** MC and **(H)** LA representative images of the fruit along with agglutination/microscopic view and graphical representation of the extent of HA.

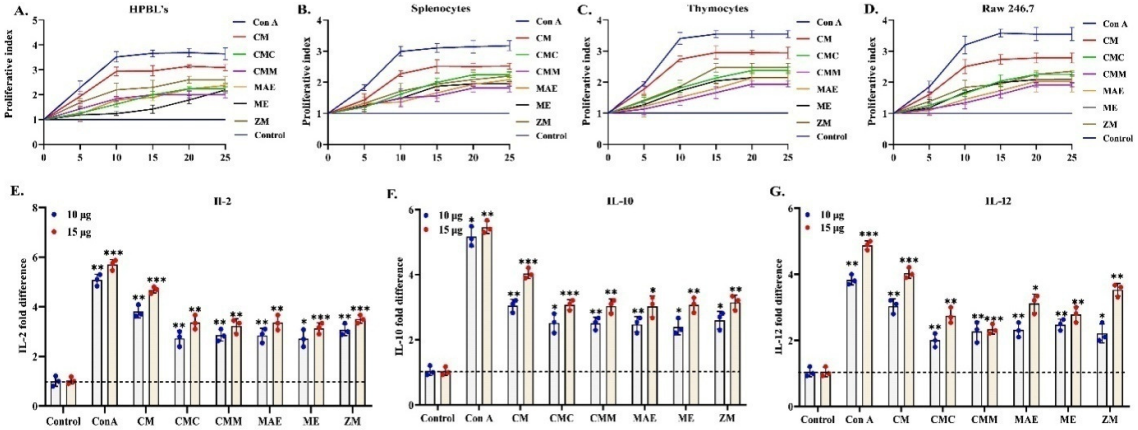

Fig. 2. Six WEF's extract proliferates immune cells through immune cytokines: Immune cells, lymphocytes, splenocytes, thymocytes and Raw 246.7cells were cultured and treated with different concentrations WEF's & Con A as a reference mitogen and MTT assay was carried out to measure the proliferation index. **(A-D)** Proliferative index of PBLs, splenocytes, thymocytes and Raw 246.7 cells: The conditioned media from WEFs treated HPBL's cells were subjected to cytokines measurement through ELISA. **(E-G)** Levels of IL-2, IL-10 and IL-12. Results are reported as the mean ± S.D. of three different determinations. Significant statistical values are expressed as *p <0.05; **p <0.01 and ***p <0.001.

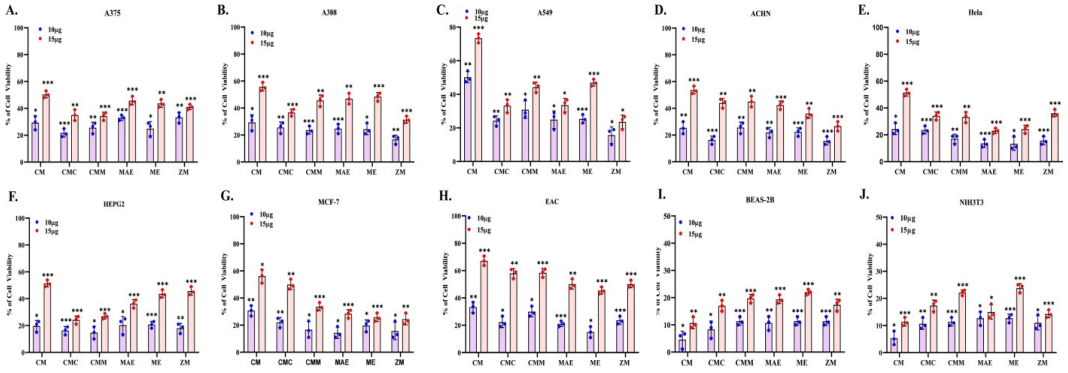

Fig. 3. Six WEF's extract induce antiproliferative activity against various cell lines: To investigate the antiproliferative effect of different WEFs, *in vitro* cultured various cell lines (A549, A388, A549, ACHN, Hela, HepG2, MCF-7, EAC and normal cell lines BEAS-2B & NIH-3T3) were treated with different WEFs extract in a dose dependent manner. The graph represents the percentage of cell viability **(A)** A375, **(B)** A388, **(C)** A549, **(D)** ACHN, **(E)** Hela, **(F)** HepG2, **(G)** MCF-7, **(H)** EAC **(I)** BEAS2B and **(J)** NIH3T3. Results are reported as the mean ± S.D. of three different determinations. Statistically significant values are *p <0.05; **p <0.01 and ***p <0.001.

Six WEF's extract induce antiproliferative activity against various cell lines: Six WEF's (0, 10, and 15µg) treated with HPBLs condition media were tested against cancer and normal cell lines to assess their antiproliferative effects. The results suggest that, among all WEF's, CM-treated condition medium from HPBLs has strong cytotoxic efficiency against A549 and EAC but not against normal cell lines, whereas other WEF's have moderate cytotoxicity against malignant cells (Fig. 3).

DISCUSSION

Wild edible fruits have recently gained recognition for their nutritional and healthful advantages, particularly associated with the immune system. These fruits which are frequently found in nature and contain a variety of bioactive components that promote overall good health. WEF's can greatly improve immune function due to their high antioxidant content, diverse vitamin profile, anti-inflammatory capabilities and natural antibacterial activities. As awareness of their advantages spreads, these underused resources may become more valuable for their contributions to health (Li *et al.,* 2016; Bellows *et al,* 2023). These fruits contain various lectins that contribute to their nutritional and medicinal properties (Souza *et al.,* 2013). These lectins play vital role in immune system modulation, antibacterial activity as well as potential anticancer efficacy. As investigation continues to investigate the full range of advantages provided by wild edible fruits and their lectins, they may become more recognized for their contributions to health and medicine (Singh *et al.,* 2014).

The results suggest that, among all WEF's, CM-treated condition medium from HPBLs has strong cytotoxic efficiency against A549 and EAC but not against normal cell lines, whereas other WEF's have moderate cytotoxicity against malignant cells (Fig. 3).

With this rationale, in current study, eight WEF's were collected from different region of Karnataka, Southern state of India. The fruits were randomly collected throughout the year at different seasons. The rational in selection of these fruits are, they are uncultivated and vast unreported traditional medicinal value. Very few studies have documented the phytoconstituents and pharmacological properties of selected eight WEF's (Dai *et al.,* 2010; Waseem *et al.,* 2018; Bidkar *et al.,* 2012; Shaik *et al.,* 2011; Prakash *et al.,* 2021; Butle *et al.,* 2021; Deshpande *et al.,* 2013; Sujono *et al.,* 2020; Upadhye *et al.,* 2021; Pandey *et al.,* 2014; Vijayvargia *et al.,* 2014; Patel *et al.,* 2014). With aim to explore their importance, a systemic investigation was carried out with experimental evidence in *in-vitro.* Our research was focused to screen for the presence of lectin in these fruits and investigate the immunomodulatory effect, particularly targeting cancer. Out of the eight fruits screened for lectin, six WEF's extract exhibits significant agglutination. Among these, CM exhibited much more significant HA activity in comparison to other fruits extracts such as CMC, CMM, MAE, ME, and ZM, which displayed moderate HA activity (Fig. 1).

Lectins from wild edible fruits have been extensively exhibited as effective immunomodulators. They can interact with glycan moieties on immune cells and enhance the immune system by increasing lymphocyte proliferation. This mitogenic action enables them to activate a wide spectrum of immune cells, so enhancing the body's defences against infections and diseases (Bah *et al.,* 2013). In contrast, our experimental data shown that selected WEF's extract induced immune cell proliferation in immune cells, particularly CM extract induced much more proliferation of immune cells compare to other WEF's extract which play a role in immunomodulation (Fig. 3). Certain plant lectins have been proved to enhance the production of cytokines, which are essential for regulating immune responses to infections and tumours. This immunomodulatory impact is predominantly supported via the interaction of lectins with glycan moieties located on the surface of immune cells, activating signal transduction pathways that improve immune function (Mishra *et al.,* 2019; Souza *et al.,* 2013). To investigate the same the lymphocytes were treated with the presence and absence of WEF's extract and supernatant was collected from the treated group as well as the untreated group to evaluate cytokine level. The result postulate that CM extract shows upregulation of immune cytokines IL-2, IL-10 and IL-12 secretion, whereas other WEF's extract exhibit moderate secretion of immune cytokines (Fig. 3).

CONCLUSION

The investigation is a novel and first time we are reporting the presence of lectin in six WEF's which were unnoticed and with high medicinal value. Among six fruits extract, lectin from CM has strong immunomodulation activity by proliferating immune cells and secreting immune cytokines. The CML induced immunocytokines were able to induce antiproliferative activity. Such diet-based lectin with immunomodulatory capacity is indeed necessary to establish therapeutic strategy against cancer. Future studies are focused to investigate the potential role of these CM with systemic investigations.

FUTURE SCOPE

Exploring the immunomodulation and antineoplastic activity of *Cordia myxa* lectin. Develop *Cordia myxa* lectin as potent therapeutic agent in cancer therapy by unravelling in detailed mechanisms of action.

Conflict of Interest. None.

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