In vitro antimitotic and cytotoxic potential of plant extracts: a comparative study of *Mucuna pruriens*, *Asteracantha longifolia* and *Sphaeranthus indicus*

Praveen Suresh Gupta and Shivani Patel

**Abstract**

**Background:** Plants have been used in alternative and traditional medicines for the cure of different types of diseases since ancient time. Secondary metabolites from natural sources play a crucial role in the treatment of various ailments. The present study carried out to investigate the phytochemical, antimitotic and cytotoxic activity of methanolic (95%) extracts of *Mucuna pruriens* seeds, *Asteracantha longifolia* seeds and *Sphaeranthus indicus* stems.

**Result:** Phytochemical analysis was performed using qualitative test to confirm the presence of phytochemical such as flavonoids, terpenoids, amino acids, cardiac glycosides, saponins, steroids, tannins, phenols and carbohydrates. The antimitotic activity was screened by using *Allium cepa* root meristematic cells. Methotrexate (0.1 mg/mL) was used as a standard. The data was analyzed by using software GraphPad Prism, Version 6.0 (GraphPad Software Inc., San Diego, CA) with one-way ANOVA. A statistical difference of $p < 0.05$ was considered significant in all cases. 

**Conclusion:** *A. longifolia* has significant amount of nearly all phytochemicals as compared to other two plant extracts. It is found that all three plant extracts showed antimitotic activity having $p$ value less than 0.05. The cytotoxicity assay revealed that all plant extracts displayed inhibition of MCF7 and A549 cells lines. *A. longifolia* showed better activity against MCF7 while *M. pruriens* possessed mild cytotoxic effect against both MCF7 and A549 cell lines.

**Keywords:** Phytochemical, Antimitotic, Anticancer, Medicinal plants

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Background

Plants have been used as an excellent source of medicine since beginning which established a foundation of traditional medicine. Such traditional medicinal plants play vital role to fulfill worldwide health-care needs nowadays and their use will increase in the future [1]. Owing to the side effect of chemical drugs, the use of medicinal plant extracts for the treatment of human diseases has greatly increased in the past few decades. Plants have antimitotic, anti-diuretic, anti-diabetic, anti-arthritis, anti-depressant, analgesic, anti-inflammatory, antioxidant, antibacterial, etc., properties due to the presence of various bioactive compounds such as phenolics, flavonoids, alkaloids, terpenes, steroids and saponins. The phytochemicals in plants act as medicine; therefore, plants have been used as a source of medicine for thousands of years [2]. Due to structural diversity of natural products, it contributes major significance in the development of number of modern drugs [3]. Among all the diseases, cancer is the major cause of death, since it is multifactorial disease, so development of cancer drug has always been a promising area in recent past. Researches on anticancer agents have become a worldwide effort in both developed and developing countries. More than 50% of the drugs for anticancer activity were obtained from natural sources or are related to them [4]. According to the World Health Organization, about 9.6 million deaths due to cancer have been reported worldwide in 2018. Approx 7.85 lakh deaths have been reported in India during 2018 [5]. This number may increase to 12.0 million by 2030 [6].

For a long period, plants and their products are being used in the treatment of cancer [7]. Exploration of traditionally medicinal plants is important on two levels: firstly, as a source of possible chemotherapeutic drugs and secondly, as an evaluate of safety for the continued use of medicinal plants [8]. The greater part of standard anticancer drugs has been derived from natural sources, based on their use in traditional medicine [9]. Cancer is uncontrolled mitotic division of cell. Use of antimitotic agent is one of the significant aspects in treatment of cancer. Antimitotic agents constitute a major class of cytotoxic drugs; these cytotoxic drugs will remain stay in cancer chemotherapy for the next future [10]. Knowledge of phytochemical constituents is requisite to understand the mechanism of bioactivity of plant extracts. Anti-oxidant compounds such as phenolic acids, polyphenols, and flavonoids scavenger free radicals such as peroxide and hydroperoxide and thus inhibit oxidative stress. The free radical assumption supported the fact that the antioxidants effectively inhibit the DNA damage and uncontrolled division of cells. Flavonoids possess broad spectrum functions including antioxidant, antimitagenic and anticarcinogenic activity [11]. Important enzymes SOD, CAT and glutathione peroxidase are involved in the clearance of superoxide and hydrogen peroxide [12]. Initially alkaloids from Vinca rosea and taxol from Taxus brevifolia were primarily used as antimitotic drugs that targeted tubulin organization and also showed the cytotoxic effect that lead to great success in cancer therapy [13]. Allium cepa root tip cell has been proved to be a reliable and cost-effective source for the detection of cytotoxic and mutagenic properties [14]. Cell lines are potential source to investigate novel compound and its effects on tumour cells [15]. Cell line acts as an excellent cancer model to evaluate in vivo anti-proliferative activities of new compound [16]. Present cancer treatments, such as chemotherapy, radiation, targeted therapy and immunotherapy, have harmful side effects. Therefore, herbal medicine has been considered as a useful choice to the presently existing therapies [17].

For this study, three least explored medicinal plants Mucuna pruriens, Asteracantha longifolia and Sphaeranthus indicus have been selected to evaluate and compare their antimitotic and cytotoxic potential against breast cancer cell line MCF7 as well as lung cancer cell line A549. Mucuna pruriens (Fabaceae) is a tropical legume and annual climbing shrubs native to Africa and tropical Asia and commonly cultivated. Various pharmacological activities like antioxidant, anti-cholesterolemic, anti-Parkinson, anti-diabetic, sexual enhancing, anti-inflammatory, antimicrobial and antivenom activities of M. pruriens have been reported. It also reveals antican-cer activities, but very few evidences have been reported [18]. Further exploration is required to investigate its antimitosis, anticancer and cytotoxic potential. It is an effort to reduce the gap in the study of Mucuna. This plant is rich in L-Dopa, very important in brain health [19]. Evaluation of phytochemical constituents and antioxidant property of Vitex doniana and Mucuna pruriens by inhibition of DPPH radical was used to evaluate their free radical scavenging activity [9].

Asteracantha longifolia (Acanthaceae) is an annual herb having good medicinal value widely distributed in India, Sri Lanka, Malaysia and Nepal and extensively used in diuretics, jaundice, kidney stone, rheumatism, liver dysfunction, urinogenital problems, etc. It is rich with steroids, alkaloids, butelin, lupeol and fatty acids also used in diabetes and dysentery [20]. Similarly, Sphaeranthus indicus (Asteraceae) is also an annual herb distributed throughout India, Sri Lanka, Africa and Australia extensively used in traditional medicine to cure various health problems like mental illness, fever, and cough [21]. Very few studies are reported on antimitosis
and cytotoxic potential of Mucuna pruriens, Sphaeranthus indicus and Asteracantha longifolia. All three plants show diverse pharmacological properties, but present studies carried out to evaluate antimitotic and cytotoxic potential of selected plants.

Methods

Collection and processing of plants samples

Samples were collected from wild area of Junagadh district, Gujarat, India (21.522° N, 70.4579° E) during October month. Plant parts (seeds of Mucuna pruriens, Asteracantha longifolia and stems of Sphaeranthus indicus) were washed and dried in shaded area for further studies. The taxonomic identification and authentication of plants were carried out by Dr. Neha Patel (Botanist), Shree M. & N. Virani Science College (Autonomous), Rajkot. Voucher specimen of M. pruriens (no: NTP/14/03/2019/8), A. longifolia (no: NTP/14/03/2019/9) and S. indicus (no: NTP/14/03/2019/10) were deposited in Institute. These dried samples were taken separately, made into powder form by fine grinding and then used in further extraction process. Allium cepa (40 ± 10 g) bulbs were purchased from the local market and stored for the study.

Preparation of plant extract

Plant materials (100 g) were soaked in 450 mL of 95% hydroalcoholic solvent (Methanol: water). This solvent system placed for 48 h at room temperature. After 48 h, solvents were filtered through Whatman filter paper no.1 separately in a beaker. The filtrate was dried in a rotary evaporator (Equitron, India) at 55°C to obtain the concentrated yield of extracts. Solvent extraction carried out by slightly modified standard procedure developed by de Mesquita et al. [22]. Modifications are done at temperature and solvent used. Sample directly dissolved in solvent; no maceration has been used for extraction. These extracts were placed in cold condition (4°C) for further studies.

Phytochemical analysis

Qualitative analysis was carried out to identify the presence of major phytochemical compound in all three plants. Different tests for the presence of flavonoids, terpenoids, saponins, steroids, tannins, phenols, carbohydrates, amino acids, cardiac glycosides and alkaloids were performed. The phytochemical analysis of plant extracts was carried out using standard procedures.

The presence of flavonoids was analyzed by lead acetate test by adding 1 mL of aqueous plant extract to 1 mL of 10% lead acetate solution, appearance of yellow colour give positive result [23]. The terpenoids were performed by Salkowski test by adding 0.5 mL of plant extract with 0.2 mL of chloroform. Add 0.3 mL conc. H₂SO₄, if reddish brown colouration observed it mean test is positive. To investigate the occurrence of amino acids, Biuret test was performed, 0.5 mL of plant extract was added with few drops of 2% (w/v) of copper sulphate, 1 mL of ethanol followed by excess of potassium hydroxide pellets [24]. The formation of pink colour in the extract layer indicates the presence of amino acids. Presence of cardiac glycosides was confirmed by Killer-Killani test; 0.5 mL plant extract was mixed with 0.08 mL glacial acetic acid along with 1–2 drops of FeCl₃ and observed for brown ring at interface. Foam test was carried out for saponins; 0.5 mL of plant extract was added with small amount of ethanol and distilled water and vortexed in graduated cylinder. The appearance of stable foam confirmed the presence of saponins in plant extract. Liebermann-Burchard test was used to analyse the presence of steroids in which 2 mL of acetic anhydride was mixed with 0.5 mL plant extract followed by 2 mL H₂SO₄ [25]. The absence of any colour change indicated the presence of steroids. The tannins were analyzed by adding few drops of 1% FeCl₃ to 0.5 mL plant extract and intense green or black colour confirmed the presence of tannins [26]. Ferric chloride test was used for estimating phenols where addition of 0.5 mL plant extract to 3–4 drops of 5 % FeCl₃ solution forms violet colour that indicated the presence of phenols [27].

The carbohydrate in extract was investigated by Molisch’s test. To 0.5 mL plant extract, 2–3 drops of 1% alcoholic α-Naphthol solution was added and formation of violet ring at junction confirmed the presence of carbohydrates. The alkaloid test was performed by four different methods and in each method 0.5 mL plant extract was used: (i) Dragendorff’s reagent (Potassium-Bismuth-Iodide solution) in which 0.5 g of bismuth nitrate was added with 10 mL of concentrated hydrochloric acid followed by completely dissolving 4 g potassium iodide in minimum water in separate beaker and observed for dark orange colour. (ii) In another method, Mayer’s reagent was freshly prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5 g) in 100 mL of water and treated with a drop or two of Mayer’s reagent and occurrence of cream coloured precipitation indicates the positive test. (iii) The Wagner’s reagent was prepared by adding 2 g of iodine and 6 g of potassium iodide in 100 mL of water [28]. (iv) Hager’s reagent was prepared by dissolving 1 g of picric acid in 100 mL of water [29].

Antimitotic assay

A modified method described by Fiskesjo (1985) was used for evaluation of antimitotic activity using Allium cepa root [30]. Allium cepa bulbs (40 ± 10 g) were germinated in water for 72 h at room temperature in dark condition. The bulbs that developed uniform root were selected for further studies (Fig. 1). These roots of
Onions were placed on beaker filled with water, methotrexate (0.1 mg/mL) and plant extracts (10 mg/mL) for 24 h. Water used for dilution as well as control and methotrexate used as a standard for study. Set up for all three plant extracts along with control (water) and standard (methotrexate) have done separately in triplicate. After 24 h, the root tips were stained by acetocarmine and observed under light microscope (Olympus CX21i, Japan) at 10× magnification using CatCam software and counted the number of dividing and non-dividing cells. The mitotic index was calculated by following formula:

\[
\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100
\]

**Statistical analysis**

The data were expressed as the mean of three replicates with standard deviation. GraphPad Prism software was used for analysis of p value. One-way analysis of variance (ANOVA) was used to analyse the significant difference between controls, standard and methanolic extracts of *M. pruriens* and *A. longifolia* seeds extracts and stems extracts of *S. indicus*. P < 0.05 was considered as significant.

**Cytotoxicity assay**

MTT assay was carried out to assess the cytotoxicity of all plant extracts on breast cancer cell line MCF7 and lung cancer cell line A549 [31]. Both cancer cell lines were purchased from National Center for Cell Science (NCCS), Pune, and the cells were maintained in MEM supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL⁻¹), in atmosphere of 5% CO₂/95% air at 37 °C.

**Preparation of samples**

For MTT assay, each extracted plant sample was weighed separately and dissolved in DMSO and the final concentration make up to 1 mg/mL using MEM. The cells were treated with varying concentrations of plant extracts sample ranging from 10 to 100 μg/mL. Cell viability was evaluated by the MTT Assay using six different concentrations of compounds. After trypsinization of cell, trypan blue assay was performed to analyse the cell viability in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 x 10^3 cells/well in 100 μl medium in 96-well microtitre plate and incubated overnight at 37 °C in 5% CO₂ incubator at 95% air. After incubation, the culture medium was discarded and replaced with 100 μl fresh medium and further incubated for 48 h. To each well, 10 μl MTT solutions (0.5 mg/mL⁻¹) were added and plates were incubated at 37 °C for 3 h. After incubation, the plates were observed for precipitates formation due to reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader (Aventor Bensphera, USA). All the experiments were performed in triplicates. The percentage growth inhibition was calculated using the following formula:

\[
\%\text{Inhibition} = \frac{100 \times (\text{Control} - \text{Treatment})}{\text{Control}}
\]

The IC_{50} value was determined by using linear regression equation, i.e. \( y = mx + c \). Here, \( y = 50 \) and \( m \) and \( c \) values were derived from the viability graph.

**Results**

**Phytochemical analysis**

Phytochemical analysis showed the presence of phyto-constituents such as flavonoids, terpenoids, saponins,
steroids, phenol, tannins, cardiac glycosides, amino acids and alkaloids in all three plant extracts as shown in Table 1. It is found that flavonoids, terpenoids, tannins and carbohydrate are present in all three plant samples. *M. pruriens* seed extracts showed higher amount of saponins, carbohydrates and alkaloids; however, steroid, phenol and amino acids were absent. *A. longifolia* seed extract has rich amount of these compounds except saponins and amino acids. *S. indicus* stem showed significantly more amount of flavonoids, tannins and carbohydrate and rest of the phytochemicals were absent. Mild amount of flavonoids were found in all the three plant extracts. *M. pruriens* is found to be rich in alkaloids in comparison to both plants. Most of initially discovered anticancer drugs were alkaloids and some of these alkaloids were used to successfully develop different chemotherapeutic drugs, such as CPT, a famous topoisomerase-I inhibitor [32]. Vinblastine is also an alkaloid which interacts with tubulin protein and affects spindle fibre organization.

### Antimitotic assay: microscopic observation

After staining, the root tip cells were observed under light microscope by using acetocarmine stain. The microscopic view of stained root tip cells at 10× is shown in Fig. 2.

The results of effect of *M. pruriens, A. longifolia* and *S. indicus* on mitotic index (MI) of *Allium cepa* root tip cells are given in Table 2.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism software. The graph showed comparative analysis of

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**Table 1** Phytochemical profile of all three plant extracts

| Phytoconstituents | Name of test     | MPS | ALS | SIS  |
|-------------------|------------------|-----|-----|------|
| Flavonoids        | Alkaline test    | +   | +++| +++  |
|                   | Lead-acetate test| −   | +++| +++  |
| Terpenoids        | Salkowski test   | +   | ++ | −    |
| Steroids          | Liebermann-Burchard test | −   | ++ | −    |
| Saponins          | Foam test        | ++  | −  | −    |
| Tannins           | Ferric chloride test | + | +++| +++  |
| Phenols           | Ferric chloride test | − | ++| −    |
| Carbohydrates     | Molisch’s test   | +++ | +  | +++  |
| Amino acids       | Biuret test      | −   | −  | −    |
| Cardiac glycosides| Killer killani test | + | + | −    |
| Alkaloids         | Dragendorff’s test| +++| ++| −    |
|                   | Mayer’s test     | ++  | ++| −    |
|                   | Wagner’s test    | ++  | ++| −    |
|                   | Hager’s test     | ++  | + | −    |

+ slightly present, ++ moderately present, +++ highly present, − absent

MPS methanolic extract of *M. pruriens* seed, ALS methanolic extract of *A. longifolia* seed, SIS methanolic extract of *S. indicus* stem

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**Fig. 2** Microscopic observation at 10× magnification of *Allium cepa* meristematic cells treated with (A) water, (B) methotrexate (standard) and (C) plant extract: (I) *M. pruriens*, (II) *A. longifolia* and (III) *S. indicus*. Arrows showing dividing cells.
mitotic index of water, methotrexate and plant extracts treated Allium cepa root tips. Extract of M. pruriens, A. longifolia and S. indicus showed significant antimitotic activity, by decreasing rate of mitosis in comparison to water. Methotrexate (0.1 mg/mL) was used as a standard and shows highest antimitotic activity. By using the p value of M. pruriens, A. longifolia and S. indicus were calculated p = 0.0001. All three values indicate significant difference between the independent groups. Thus, all three plants displayed significant antimitotic activity which indicates its use as a potent antimitotic agent (Fig. 3).

Cytotoxic assay
The IC50 values of plant extracts treated with MCF7 and A549 cells is shown in Table 3. A. longifolia showed better activity on MCF7 with IC50 of 12.32 μg/mL and the plant S. indicus showed the lesser activity on MCF7 with IC50 of 185.56 μg/mL. IC50 is the value at which growth of 50% cells inhibited. IC50 values of all three plants samples on MCF7 and A549 cell lines were calculated which are shown in Table 4 and Fig. 4.

Discussion
Although various reports are available on M. pruriens, A. longifolia and S. indicus, there are very few reports on antimitotic and cytotoxic potential of these plants. Due to potential antimitosis activity, these plants might be used in exploration of drug for the treatment of cancer. Cancer is caused due to many factors which affect multiple pathways related to cancer. More than two hundred types of cancers are reported so use of multiple cell line is recommended for study. Cell line shows different sensitivity against the drug or molecule at different concentration.

Study targeted the exploration of all three plants for their antimitotic and cytotoxicity potential. Allium cepa used for antimitosis assay and two different cell lines used for cytotoxicity study. Investigation of in vitro cytotoxicity of plant extracts is preliminary step of research for anticancer compound [33]. Similar approach has been used to investigate cytotoxic and antimitotic potential of many plants. Evaluation of in vitro cytotoxicity which cause DNA damage in mice was studied by using extract of Xanthium strumarium L. [34]. Potential genotoxic activity in Pterocaulon polystachyum through A. cepa test detected an antimitosis property related to increasing concentrations of aqueous extracts [35].

| Treatments               | Mitotic index (MI) % M. pruriens | A. longifolia | S. indicus |
|--------------------------|----------------------------------|--------------|-----------|
| Water (control)          | 90.81 ± 1.26                     | 86.24 ± 2.18 | 83.63 ± 1.14 |
| Methotrexate (standard)  | 14.61 ± 0.91                     | 14.31 ± 1.9  | 15.26 ± 0.99 |
| Plant extracts           | 60.78 ± 1.25                     | 43.96 ± 1.87 | 51.89 ± 1.22 |

All data expressed as mean values ± SD (n = 3) represented by error bars.

| Concentration(μg/mL) | M. pruriens MCF7 | A549 | A. longifolia MCF7 | A549 | S. indicus MCF7 | A549 |
|----------------------|------------------|------|-------------------|------|----------------|------|
| 5                    | 69.92            | 86.94| 80.09             | 56.33| 66.95          | 70.62|
| 10                   | 75.85            | 65.11| 71.19             | 51.23| 60.17          | 60   |
| 25                   | 98.31            | 30.82| 0                 | 47.15| 58.48          | 40.82|
| 50                   | 0                | 37.35| 0                 | 55.52| 56.36          | 33.07|
| 100                  | 0                | 32.86| 0                 | 43.07| 57.63          | 42.25|

Table 2 Effect of M. pruriens, A. longifolia and S. indicus on mitotic index (MI) of Allium cepa root tip cells

Table 3 Percentage cell viability of MCF7 and A549 cell line after treatment with M. pruriens, A. longifolia and S. indicus extracts

![Fig. 3](image-url)
cytotoxic and antimitotic activities of *Solanum nigrum* has been explained by using *Allium cepa* root tip assay and cancer chemo-preventive activity using MCF7 [36]. It has been reported that methanolic extracts of *M. pruriens* leaves have several biochemical and physiological activities, and it also contain pharmaceutically important compounds [37]. *M. pruriens* seeds have been used for the study on antitumor and in vivo antioxidant activity against *Ehrlich ascites* carcinoma in Swiss albino mice [38]. Preparations made from *Mucuna* seeds are used for several disease caused by free radicals such as ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders [39]. Anticancer activity of *A. longifolia* extract has been evaluated which results in a significant decrease in the tumour size of DMBA-induced mammary tumour in mice. It also decreases the levels of lipid peroxidation and significantly increased the levels of superoxide dismutase and catalase [40]. It is also reported that it promotes the antitumor activity against hepatocarcinogenesis in rats [41]. It is also observed that *A. longifolia* is rich in phytochemical in compare to *M. pruriens* and *S. indicus*. A flavone glycoside is present in stem of *S. indicus*. Flavone glycoside also called baicalin which is a potent compound against certain cancer has been reported. Flavone glycoside suppresses the cell cycle progression. It significantly decreases cell cycle associated protein resulting in the decrease rate of mitosis [42]. Two phytochemicals β-sitosterol and 7-hydroxyfrullanolide were isolated from *Sphaeranthus indicus* induce apoptosis by inducing loss of mitochondrial membrane potential and also induce DNA fragmentation in human leukaemia HL-60 cell line [43].

### Table 4 IC50 value of all three plant samples on MCF7 and A549 cell lines

| Plant extracts | IC50 (μg/mL) |
|----------------|-------------|
|                | MCF 7       | A549       |
| *M. pruriens*  | 36.74       | 39.42      |
| *A. longifolia*| 12.32       | 44.97      |
| *S. indicus*   | 185.56      | 35.43      |

**Fig. 4** Graph showing IC50 of extracts of (I) *M. pruriens*, (II) *A. longifolia* and (III) *S. indicus* on (A) MCF7 and (B) A549 cell lines
Conclusion
Present study on antimitotic and cytotoxic activity of M. pruriens, A. longifolia and S. indicus suggests that all three plants have potential antimitotic and cytotoxic properties. These activities showed the presence of major bioactive compounds and biochemical constituents in these plants due to which these plants might be used as potential source of drug to treat cancer. Further studies comprehend the specific effect of bioactive compound.

Abbreviations
ANOVA: Analysis of variance; CAT: Catalase; CPT: Camptothecin; DMBA: 7, 12-Dimethylbenz (a) anthracene; DMISO: Dimethyl sulphoxide; DPH: 2, 2- Diphenyl-1-picryl-hydrazyl; FBS: Foetal bovine serum; IC: Inhibitory concentration; MEM: Minimal essential medium; MI: Mitotic index; MTT: 3-(4,Dimethylbenz (a) anthracene; DMSO: Dimethyl sulphoxide; DPPH: 2, 2-

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Plant authentication
Herbarium Authentication Certificate (Ref: Cook Flora of Gujarat State, India) of the plants used in this study has been attached with the manuscript.

Authors’ contributions
We declare this work was done by the authors mentioned in this manuscript. PSG designed the study and carried out laboratory work. SP supervised, helped in data analysis and revised and corrected the final manuscript. All authors read and approved the final manuscript for submission.

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