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Research Paper

PHYSICOCHEMICAL AND PHYTOCHEMICAL CONTENTS OF THE LEAVES OF *Acrostichum aureum* L.

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Abstract

The physicochemical parameters are mainly used in judging the purity of the drug. Hence, in the present investigation, moisture content, total ash, water-soluble ash, acid-soluble ash, sulphated ash and different solvent extractive values are determined. Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. In the present study, chief phytoconstituents of the *Acrostichum aureum* L (Fern) medicinal plant of the Pteridaceae family were identified to relate their presence with bioactivities of the plants. These research findings highlight that methanolic extracts of *A. aureum* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *A. aureum* leaves holds the great potential to treat various human diseases and has profound medical applicability. Fluorescence analysis of powder under visible light and UV light helps establish the purity of the drug. Hence, fluorescence analysis of leaf powder is undertaken.

Key words: *Acrostichum aureum* L., Pteridophytes, Physicochemical, Phytochemical Screening, Fluorescence Analysis.

INTRODUCTION

The World Health Organization (WHO) estimates that 80% of people in developing countries depend on traditional medicine for their health needs, and 85% of traditional medicine involves the use of plant extracts. Ferns are generally used in traditional medicine for the cure of many deadly diseases like skin problems, wounds, cough and reproductive problems as well as to make insect repellent [1, 2]. A wide range of

medicinal ferns like *Adiantum capillus-veneris*, *Cheilanthes albomarginata, Asplenium nidus*, *Ceratopteris thalictroides* including, *Acrostichum aureum* exist in Asia [3-5]. *A. aureum* is a member of Pteridaceae which is commonly known to the locals as the Swamp Fern or Mangrove Fern. It is an evergreen shrub, found in a hostile environment. Plant species that thrive in a hostile environment replete with bacteria, fungi or virus synthesize defensive natural products against these pathogens, which may also exhibit bactericidal, fungicidal or virucidal activity in the human system [6]. Several studies have reported the traditional use of *A. aureum's* rhizome for curing wounds, non-healing ulcers, boils, syphilitic ulcers, sore throat, chest pains, elephantiasis, purgative, febrifuge, cloudy urine in women, and rheumatism in Malaysia [7], Bangladesh [8], and Yap islands and Micronesia [9]. Rhizome paste is applied to heal the wounds and boils. It is also used as anthelmintic, vulnerary, healing inveterate ulcers, and bladder complains in China. Fertile fronds are used for syphilitic ulcers in Borneo. Fronds are used as an antifungal agent [10]. In Bangladesh, preparations from rhizomes and leaves of *A. aureum* are used to treat wounds, peptic ulcers and boils [11]. The native people of Costa Rica use leaves as emollients, whereas, the Cuna people (Panama and Colombia) use the young fiddleheads to extract fish bones from the throat and as a medicinal bath for infants [12]. The crude extract of a Japanese *A. aureum* specimen is reported to possess anti-oxidant, tyrosinase inhibiting activity [13], while a Hainan specimen reported anti-tumour activity against cervical cancer cell line [14]. Uddin *et al*. [15] reported the cytotoxic effect of water and methanol extracts from a Bangladeshi specimen of *A. aureum* leaves on gastric, colon and breast cancer cells.

An understanding of the chemical constituents of plants is a prerequisite for their use in medicine and also for the synthesis of complex chemical substances. Correlation between the phytoconstituents and the bioactivity of the plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world. Thus, the present study deals with the physicochemical and phytochemical screening of *Acrostichum aureum* L., leaves.

MATERIAL AND METHODS

Collection and Identification of the Plant Material

The leaves of *Acrostichum aureum* L. were collected from the Puthalam, Kanyakumari District, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. A voucher specimen (VOCC/VV/Bot/11) was also deposited in the PG & Research Department of Botany, V.O. Chidambaram College, Tuticorin.

Preparation of *A. aureum* Leaf Powder

Dry conditions are essential to prevent the formation of artefacts as a result of microbial fermentation and subsequent degradation of the plant metabolites. The plant materials are cut or sliced into small pieces to facilitate homogenous drying and prevented from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites resulting in the formation of artefacts. Hence in the present study, the leaves of *A. aureum* were cut into small pieces and were dried in shade and then powder with a mechanical grinder. The powder was passing through sieve number 75 and stored in a labelled air-tight container for further studies.

Physicochemical Studies

Physicochemical studies include loss on drying (moisture content), ash value and extractive value to determine the quality and purity of the powder of the leaves of *A. aureum*.

Moisture (Loss on Drying)

About 3g of the air-dried sample was weighed (W_b), into a pre-dried and weighed (W_a) tarred porcelain crucible. The sample was dried in an oven at 100-105°C until two consecutive weighing's (W_c) do not differ by more than 5mg. The moisture content of the sample was calculated about crude air-dried drug [16].

$$\text{Moisture (\%)} = \frac{(W_b - W_C)}{(W_b - W_a)} \times 100$$

Ash Values

Total Ash Value

A silica crucible was heated to redness for 10min and cooled in a desiccator and weighed (W_1). About 3g of the ground air-dried sample was transferred to the crucible and weighed along with the contents accurately (W_2). The sample was ignited gradually in an electrical muffle furnace, increasing the heat to 500–600°C until it is white,

indicating the absence of carbon. It was cooled in desiccators and reweighed (W_3) [17]. Total ash content was calculated as in equation

Total ash (%) = $\frac{(W_3 - W_1)}{(W_2 - W_1)}$ x 100

Acid-insoluble Ash (Silica & Sand content)

10ml of 2M HCl was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5min. The watch-glass was washed with 5ml of hot water and the washings were added to the crucible. The insoluble matter was filtered on an ashless filter paper and washed with hot water until the filtrate is neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight (W_4). The residue was allowed to cool in desiccators for 30min and then weighed [17]. Acid-insoluble ash content was calculated as in equation

Acid-insoluble ash (%) = $\frac{(W_4 - W_1)}{(W_2 - W_1)}$ x 100

Water Soluble Ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5min. The insoluble matter was collected on an ashless filter paper. The filter was washed with hot water and then ignited in a crucible for 15min at a temperature not exceeding 450°C. The residue was allowed to cool in desiccators for 30min, and then re-weighed (W_5) [17], calculations were done according to equations

Weight of residue, W_6 (g) = $W_5 - W_1$

Weight of ash W_7 (g) = $W_3 - W_1$

Water-soluble ash (g) = $W_7 - W_6$

Water-soluble ash (%) = $\frac{(W_7 - W_6)}{(W_1)}$ x 100

Sulphated Ash

A silica crucible was heated to redness for 10min, allowed to cool in desiccators and weighed (W_a). 1g of substance was accurately weighed and transferred to the crucible and weighed along with the contents accurately (W_b). It was ignited gently at first until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ± 25°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, and a few drops of concentrated sulfuric acid were added and heated. Ignited as before,

allowed to cool, and weighed (W_c) [17]. The operation was repeated until two successive weighing does not differ by more than 0.5mg. Calculate the percentage of sulphated ash concerning the air-dried drug.

Sulphated ash (%) = $\frac{(W_c - W_a)}{(W_b - W_a)}$ x 100

Extractive Values

The extractive values of leaves of *A. aureum* in various solvents like petroleum ether, benzene, chloroform, ethyl acetate, ethanol, methanol and water were determined by employing the method of analysis described in Pharmacopoeia of India [17].

About 5g of air-dried leaf powder was taken in a stoppered flask. 100ml of the respective solvent was added, shaken well and allowed to stand for 24h with occasional shaking. Then the content was filtered. 50ml of the filtrate were pipette out into a clean, previously weighed china dish and evaporated on a water bath. Finally, it was dried at 105ºC in an oven, cooled in a desiccator and weighed. The percentage of solvent-soluble extractive concerning the air-dried sample was calculated.

Preliminary Phytochemical Screening

Preparation of Plant Extract

The coarse powder was subjected to extraction in 250ml each of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents separately. The coarse powder (10g) of the leaf was weighed and put into the brown glass bottles. Then the solvents were added to it. Then the bottles were sealed with aluminium foil and kept in laboratory shaker at room temperature, and the bottles were shaken (130-140rpm) for one week. Finally, the extract was filtered through many layers of muslin cloth for coarse filtration. The coarse filtrate was then filtered through Whatman number 1 filter paper. The obtained filtrate was evaporated in a vacuum rotary evaporator under reduced pressure at 40ºC until the filtrate was reduced to one-third of the starting filtrate volume, collected in the petri dish and dried at room temperature. The dried extract from the petri dish was scraped and transferred to Eppendorf tube. A part of dry extracts powder was re-dissolved in 50ml of dimethyl sulfoxide (DMSO) and was stored in stopper glass bottles and another part was kept as such in air-tight bottles at 4ºC for further analysis.

Phytochemical Screening

The phytochemical screening gives a general idea regarding the presence of different compounds possessing therapeutic values. The different solvent extracts of *A. aureum* leaf were used for screening the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catechin, sugar and terpenoids according to standard procedures of Harborne [16], Brindha *et al* [18], Trease and Evans [19] and Sofowara [20].

Screening for Alkaloids (Dragendroff's test)

2ml of the extract was mixed with 8ml of 1% HCl, warmed and filtered. Then the filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

Screening for Steroids (Liebermann Burchard test)

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of the brown ring at the junction indicates the presence of phytosterols.

Screening for Coumarin

2ml of the extracts was taken in test tubes. The mouth of the tube was covered with filter paper treated with 3ml of 1N NaOH solution. The test tube was placed for a few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Screening for Tannins

50mg of various solvent extract powder was dissolved in 10ml distilled water and filtered. 1% aqueous iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Screening for Saponin

50mg of the various solvent extract powder was boiled in distilled water in a test tube in boiling water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The

frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Screening for Flavonoids (Shinoda Test)

To the extract solution (5ml), added few fragments of magnesium ribbon and concentrated HCl dropwise. The appearance of red or orange-red colour indicates the presence of flavonoids.

Screening for Quinone

1ml of the extract was mixed with 1ml of concentrated H_2SO_4. The appearance of red colour shows the presence of Quinone.

Screening for Anthroquinone (Borntrager's test)

50mg of extract powder was taken into a dry test tube and 5ml of chloroform was added and shaken for 5min. The extract was filtered through Whatman No 1 filter paper and the filtrate was shaken with an equal volume of 10% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicates the presence of anthroquinone.

Screening for Phenols

The extract powder (50mg) was dissolved in 5 ml of distilled water. To this few drops of 10%, ferric chloride solution was added. The appearance of a blue or green colour indicates the presence of phenol compounds.

Screening for Protein

The extract powder (50mg) was dissolved in 10ml of distilled water and filtered through Whatman No. 1 filter paper. To the filtrate, 1ml of 40% NaOH was added. Then, 1 or 2 drops of 2% copper sulfate solution were added. The appearance of violet colour indicates the presence of proteins.

Screening for Carbohydrates (Molisch Test)

To 2ml of extracts, 3 drops of α-naphthol (20% in ethanol) was added. Then 1ml of concentrated sulphuric acid was added along the side of the test tube. Reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Screening for Glycosides (Borntrager's test)

Extract powder (50mg) was mixed with concentrated H2SO4 (5ml.), then it was heated for 3 minutes, thereafter it was filtered after that filtrate was mixed with 0.5ml of 10% NaOH and allowed to stand for 3 minutes. The appearance of a reddish-brown precipitate indicates the presence of glycosides.

Screening for Reducing Sugar

For the presence of reducing sugars in the extract, Fehling test was performed. An amount of 50mg of the extract powder was taken and added it to the equal volume of boiling Fehling solutions (A and B) in a test tube. A brick- red precipitates indicate the presence of reducing sugar.

Screening for Terpenoids (Salkowski test)

5ml of the various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3ml concentrated sulfuric acid (H_2SO_4). A layer of the reddish-brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

UV Fluorescence analysis

Take about 0.5g of leaf powder into clean and dried test tubes. To each tube 5ml of different organic solvents like distilled water, 1N HCl, glacial acetic acid, 1N HNO3, liquid ammonia, 5% Ferric chloride, 5% iodine, methanol, petroleum ether, chloroform, 50% sulphuric acid, 40% NaOH and lead acetate were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible daylight and UV light of short wavelength (254 nm) and UV light of long-wavelength (365 nm) for their characteristic colour [21].

RESULTS AND DISCUSSION

The dry powder is evaluated for its physicochemical parameters like moisture content, total ash, water-soluble ash, acid-soluble ash, sulphated ash and different solvent extractive values (Table 1). The physicochemical parameters are mainly used in judging the purity and quality of the drug. Moisture is one of the major factors responsible for the deterioration of drugs and herbal formulations. The moisture promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. This study recorded moisture content of 9.25% which is deemed to be good as the water content in herbal drugs should not be greater than 14% (www.intechopen.com).

A high ash value is indicative of contamination, substitution or adulteration by minerals. The residue remaining after incineration of plant material is the total ash or ash value. Ash value represents both physiological ash and non-physiological ash. Physiological ash is derived from plant tissue due to biochemical processes while non-physiological ash consists of residue of the extraneous matter (such as sand, soil etc.) deliberately or

non-deliberately adhering to plant sample itself. Physiological ash gets dissolved in the dilute acid; while, some of the non-physiological ash remains undissolved. Total ash may compose of carbonates, phosphates, nitrates, sulphates, chlorides, and silicates of various metals which are taken up from the soil or environment [22]. In the present investigation, the total ash content of *A. aureum* leaf is found to be 7.10%, which is less than the maximum acceptable limit of total ash (14%) recommended by European Pharmacopoeia [23].

Table 1 Physicochemical constants of *A. aureum* leaf

Constants	**Percentage**
Moisture contents	9.25 ± 0.33
Total ash contents	7.10 ± 0.05
Water-soluble ash	2.41 ± 0.06
Acid soluble ash	1.69 ± 0.06
Sulphated ash	1.24 ± 0.12
Extractive values	
Petroleum ether	2.6 ± 0.04
Benzene	2.2 ± 0.03
Ethyl acetate	1.2 ± 0.03
Ethanol	5.2 ± 0.03
Methanol	4.6 ± 0.05
Water	6.7 ± 0.02

Acid insoluble ash is a part of total ash and measures the amount of silica present especially as sand and siliceous earth in the samples. The values also indicate the magnitude of presence of oxalates, carbonates, phosphates, oxides and silicates. Therefore, the values are indices of excellence of herbal remedies. Water-soluble ash is the part of the total ash content, which is soluble in water. This study shows 2.41% of water-soluble ash in *A. aureum* leaves.

Preliminary phytochemical screening of plants is important in the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to the discovery of new drugs. In the present study, the presence of fourteen phytochemicals was screened in the petroleum ether, benzene, chloroform,

ethyl acetate, ethanol and methanol extracts of *A. aureum* leaf and their results are shown in Table 2.

Table 2 Preliminary phytochemical screening of *A. aureum* leaf

Phytochemical	Name of the extract				
	Petroleum ether	Benzene	Ethyl acetate	Ethanol	Methanol
Alkaloids	-	-	-	-	-
Steroids	-	+	+	+	+
Coumarins	-	-	-	-	-
Tannins	-	-	-	-	-
Saponins	+	-	-	+	+
Flavonoids	-	-	+	-	+
Quinone	-	-	-	-	-
Anthroquinones	-	-	-	-	-
Phenols	+	+	+	+	+
Proteins	+	+	+	+	+
Carbohydrates	-	-	-	-	-
Glycosides	+	+	+	+	+
Reducing Sugars	-	-	-	-	-
Terpenoids	+	-	-	+	+

+ indicates the presence of the phytochemical; – indicates the absence of the phytochemical

Presence or absence of certain important bioactive compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act like dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. In India, traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds even by pharmaceutical companies may lead to large scale exposure of humans to natural products. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular

medicine. Hence, in the present study, the crude extracts obtained by petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol solvents were screened for the presence of phytochemicals.

The petroleum ether extract showed the presence of saponins, phenols, proteins, glycosides and terpenoids. The benzene extract showed the presence of steroids, phenols, proteins and glycosides. The ethyl acetate extract showed the presence of steroids, flavonoids, phenols, proteins and glycosides. The ethanol extract showed the presence of steroids, saponins, phenols, proteins, glycosides and terpenoids. The methanol extract showed the presence of steroids, saponins, flavonoids, phenols, proteins, glycosides and terpenoids. Among the phytochemicals, phenols and glycosides were detected in all the presently investigated solvent extracts.

These research findings highlight that methanolic extracts of *A. aureum* leaf had the highest number of phytochemicals compared to other solvent extracts. Hence, methanolic extracts of *A. aureum* leaf holds the great potential to treat various human diseases and has profound medical applicability. Smitha and Vadivel [5] also reported that the methanolic extracts of *Ceratopteris thalictroides*, a pteridophyte, also had the highest number of phytochemicals. The presence of steroids, saponins, flavonoids, phenols, proteins, glycosides and terpenoids in methanolic extracts of *A. aureum* leaf signals their therapeutic potential. Hossaini *et al* [24] reported that ethanolic extract of *A. aureum* root contains glycosides, saponins, flavonoids, steroids, fatty acids and long-chain hydrocarbon compounds.

Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic [25]. Saponins are bitter in taste and recent years, they have received considerable attention because of their various biological activities including hepatoprotective, anti-ulcer, anti-tumour, antimicrobial, adjuvant and anti-inflammatory activities. Saponins have health benefits such as cholesterol-lowering and anticancer properties [26]. Recent research has established saponins as the active components in many herbal medicines [27] and highlighted their contributions to the health benefits of foods such as soybeans [28] and garlic [29]. The presence of these compounds, therefore, suggests the good pharmacological potential for *A. aureum* leaf.

Flavonoids are secondary metabolite known to rich in pharmacological properties such as anti-oxidative, anti-fungal, anti-inflammatory and diuretic actions. Flavonoids are considered favoured bio compounds as chemotaxonomic markers in plants because

they show large structural diversity and are chemically stable [30]. The flavonoids extracted from ferns have shown promising potential because of their anti-cancer, anti-microbial [31], anti-oxidant [32] and anti-inflammatory activities [33] of the potential use in treating diabetes [34]. Flavonoids derived from *Cheilanthes tenuifolia* (fern) possess potent anti-cancerous, anti-bacterial, anti-oxidant activities that are responsible for their chemopreventive potential against bacteria [35]. Phenolics have biological and pharmacological properties such as anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities [36].

Fluorescence is an important phenomenon displayed by various phytoconstituents present in plant materials. Some show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products, which do not visibly fluoresce in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents and chemicals though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation [37-39]. The results of the fluorescent analysis of leaf powder of polyherbal formulation were depicted in Table. 3.

Table 3 Fluorescence analysis of *A. aureum* leaf powder

Treatments	Visible light	UV light	
		254nm	365nm
Powder as such	Brown	Green	Dark brown
Powder + 1N HCl	Brown	Green	Dark brown
Powder + Glacial acetic acid	Light brown	Light green	Dark brown
Powder + 1N HNO_3	Brown	Green	Black
Powder + Ammonia	Light brown	Light green	Brown
Powder + Ferric chloride	Light brown	Light green	Brown
Powder + 5% iodine	Yellowish-brown	Light green	Black
Powder + methanol	Yellowish-brown	Green	Black
Powder + petroleum ether	Brown	Dark brown	Black

Powder + chloroform	Yellowish-brown	Green	Brown
Powder + 50% H_2SO_4	Brown	Dark brown	Black
Powder + 40% NaOH	Yellowish-brown	Green	Brown
Powder + lead acetate	Yellowish-brown	Dark green	Brown

The fluorescence analysis of leaf powder of *A. aureum* showed green colour under UV light of short wavelength (254nm) when treated with 1N HCl, IN HNO_3, methanol, chloroform, and 40% NaOH, as well as, when the powder is used as such. The dark brown colour was observed under UV light of long wavelength (365nm) when the leaf powder was treated with 1N HCl and glacial acetic acid, as well as, the powder without any chemical treatment. Brown colour was observed under visible light when the leaf powder was treated with 1N HCl, 1N HNO_3, petroleum ether and 50% sulphuric acid, as well as, the powder without any chemical treatment. Various colours like light brown, yellowish-brown, light green, dark green, dark brown and black were also observed under different light conditions. The results of the fluorescent analysis of leaf powder of *A. aureum* showed characteristic colouration in treatment with various chemical reagents.

CONCLUSION

Since the plant *A. aureum* has been used in the treatment of different ailments, the medicinal roles of this plant could be related to identifying bioactive compounds. The presence of phytoconstituents, such as phenols and flavonoids in plants, indicates the possibility of antioxidant activity and this activity will help in preventing several diseases through free radical scavenging activity [4]. The present analyses suggest that *A. aureum* (fern) contains potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. Thus, it's also adding new compounds to the ever-increasing canvas of secondary metabolites acting as fountains of health. The fluorescent analysis of powdered drug plays an important role in the determination of the quality and purity of the drug.

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