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Research Article

ANTI DEPRESSANTAND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *ASPARAGUS RACEMOSUS* SEEDS

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ABSTRACT

Objective:*Asparagus Racemosus*has been referred in Indian traditional medicine system (Ayurveda) for treatment of various diseases. In the present study extracts of *Asparagus Racemosus*studied for its antidepressant activityin animal models of depression and invitro antioxidant activity.[1]
Materials and methods:Methanolic extracts of complex product prepared from dried seeds ofplant*Asparagus Racemosus*. In the present study, the antidepressant effect of *Asparagus Racemosus*was examined using two behavioural models, the forced swim test (FST) in rats and tail suspension test (TST) in mice and one invitro model such as estimation of Dopamine levels in rat brain[2].DPPH & Nitric oxide radical scavenging activity models were selected for antioxidant activity.
Results:The *In-vitro*antioxidant studies such as DPPH activity and Nitric oxide radical scavenging activity shows the satisfactory results, the concentration of extract increases the percentage inhibition of DPPH and Nitric oxide radical scavenging activity also increases, so they shows the Dose dependent action.InForcedSwimTest&TailSuspensionTest demonstratedadosedependant, statistically significantreductioninduration of immobility that was comparable to Imipramine (20mg/kg).
Conclusion:Theeffectof200mg/kg of *Asparagus Racemosus* wasbetterthan20 mg/kg Imipramine. Theeffectof100mg/kg of *Asparagus Racemosus*wassignificantwhencomparedtovehicletreatedgroup. In *in-vitro* study, *Asparagus Racemosus*in the doses of 100mg/kg and 200mg/kg showed increased levels of Dopamine when compared to that of normal. Plant extract at dose of 200 mg/kg showed increased levels of Dopamine, which is nearly equal to that of Standard.

Keywords: *AsparagusRacemosus,*Antioxidant,Antidepressant ,Methanolic extract

INTRODUCTION

This study was to prepare the extract of *Asparagus racemosus* seeds using Methanol as solvent and to study the antioxidant activity of Methanolic seed extract of *Asparagus racemosus using* following *in-vitro* models DPPH radical scavenging activity and Scavenging of nitric oxide radical and To study the antidepressant activity of Methanolic seed extract of *Asparagus racemosus* using following *in-vitro* and *in-vivo* animal models in mice by Estimation of Dopamine levels in rat brain (*in-vitro*),Forcedswimtest (*in-vivo*),Tailsuspensiontest (*in-vivo*).

Depression is an extremely common psychiatric condition, about which a variety of neurochemical theories exist and a number of synthetic antidepressant drugs are available in practice, however their effectiveness does not hold true with the entire range of population suffering from this disorder. Moreover the side effects and the drug interactions are major restrictions in its clinical utility. On the other hand, herbal medicines are widely used across the globe due to their wide applicability and therapeutic efficacy coupled with least side effects, which in turn has accelerated the scientific research regarding the antidepressant activity Antidepressantsreducetheimmobilitytimewhentheratisreplacedinthecylinder24 hours followingtheinitialexperience.Asingletestsessionwithoutapre-swimsessionisusuallycarriedoutinmice.Thesymptomsofdepressionincludeemotionalandbiologicalcomponent.Emotionalsymptoms include Misery,apathyandpessimism,Lowself-esteem:feelingsofguilt,inadequacyandugliness,Indecisiveness,lossofmotivation, Biologicalsymptoms include Retardationofthoughandaction,Lossoflibido,Sleepdisturbanceandlossofappetite.[3,4]

Asparagus racemosus(A. racemosus) belongsto family Liliaceae and commonly known as Satawar,Satamuli, Satavari found at low altitudesthroughoutIndia.Asparagusracemosus is a species of asparagus common throughout Sri Lanka, India and the Himalayas. It grows one to two metres tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1,300–1,400 metres elevation.[5]

Scientific classification
Kingdom: Plantae
Clade: Angiosperms
Clade: Monocots
Order: Asparagales
Family: Asparagaceae
Subfamily: Asparagoideae
Genus: Asparagus
Species: A. racemos

Therapeutic use of Asparagus racemosus include cooling and bitter herb is also known for it's anti-inflammatory qualities and may be used in infections such as cystitis and dysentery. Shatavari's mild diuretic action addresses the need in bladder infections, an antacid and demulcent. Satavari is effective in ulcers and hyperacidity and its cooling action works on chronic fevers, rheumatism, inflamed membranes of the lungs, Stomach, Kidneys and Sexual organs.[6]. It also used as a nervinetonic,Antilithiaticeffects,Antioxidant effects[7],Antineoplastic activity,Antitussiveeffect,,Antidepressantactivity,It helps with nervousness, pain, restless sleep, disturbing dreams and people with weak emotional and physical heart[9]. The dried roots of the plant are used asdrug. The roots are said to be tonic and diuretic and galactgogue, the drug has ulcer healing effect,probably via strenthening the mucosal resistance or cytoprotection. It has also been identified as one of the drugsto control the symotoms of AIDS.

MATERIALS AND METHODS

Extraction

The Dried seeds of were collected and authenticated.Thepowderedmaterialwassubjectedtobatchextractioninsoxhletapparatus. ThesolventsusedisMethanol.The powdered material of *Asparagus racemosus*seedswere evenly packed in Soxhletextractorforextractionwithsolvent.Thetemperaturewasmaintainedonan electricheatingmantlewiththermostatcontrol.Appearanceof

colorlesssolventinthe siphontubewastakenastheterminationofextraction.[17].Theextract swerethenconcentratedbydistillingthesolvent.Theconcentratedextracts wereevaporatedon awaterbath(40-50^{0}c)to dryness.Each extractwas weighed and percentage yield was calculated. Thecolor and consistency of theextractswerenoted.

***IN-VITRO* STUDIES**

ANTI-OXIDANTSTUDIES

DPPH radical scavenging activity

DPPH scavenging activity was measured by Spectro photometric method to an ethanolic solution of DPPH (200 µM), 0.05 ml of extract dissolved in ethanol was added at different Concentrations (10-100 µg/ml). An equal amount of ethanol was added to the Control.After20minthe decrease in absorbance of test mixtures (due to Quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition was calculated.[46]

Scavenging of nitric oxide radical - Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction. Sodium nitroprusside(5 mM) in standard phosphate buffer solution was incubated with different concentrations(10-100µg/ml)of the Methanolic extract dissolved in phosphate buffer(0.025 M; pH : 7.4) and the tubes were incubated at 25°C for 5 hr. Control experiments without the test compounds but with equivalent amount so buffer were conducted in an identical manner. After 5hr, 0.5ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2%Ophosphoric acid and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm.[47]

ANTI DEPRESSANT STUDIES

ESTIMATION OF DOPAMINE LEVELS IN RAT BRAIN: [48]

Preparation of tissue extract

- On the day of experiment rats were sacrificed, whole brain was dissected out and separated the subcortical region (including the striatum).
- Weigh a specific quantity of tissue and was homogenized in 3 ml HclButanol in a cool environment.
- The sample was then centrifuged for 10 min at 2000 rpm.
- 0.8 ml of supernatant phase was removed and added to an eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M Hcl.
- After 10 min, shake the tube and centrifuged under same conditions to separate two phases.
- Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay

- To 0.02ml of the Hcl phase, 0.005 ml 0.4 ml Hcl and 0.01ml Sodium Acetate buffer (pH 6.9) was added, followed by 0.01 ml iodine solution for oxidation.
- The reaction was stopped after 2 min by the addition of 0.1ml sodium thiosulphate in 5 M Sodium hydroxide.
- 10 M Acetic acid was added 1.5 minute later. The solution was then heated to 100oC for 6 min.
- When the samples again reach room temperature, excitation and emission spectra were read (330 to 375 nm) in a spectrofluorimeter.
- Compare the tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank).
- Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine).
- Internal reagent standards were obtained by adding 0.005 ml bi - distilled water and 0.1 ml HclButanol to 20 ng of dopamine standard.

STATISTICAL ANALYSIS

Resultswereanalyzedfor statistical significance byANOVA followed by Dunnet'smultiple comparisontests.Values*P*<0.05&belowwereconsideredsignificant.

IN VIVO STUDIES

EXPERIMENTAL ANIMALS

Adult Wistar albino rats (150-180 g)/ Swiss albino mice (25-30 g) of either sex were kept under standard environmental conditions of room temperature (220 ±20C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six mice per cage) and feeded and water *adlabidum.*

All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee.

ANTI DEPRESSANT ACTIVITY

FORCEDSWIMTEST

GroupI : Control (normal saline-5ml/kg)
GroupII : Imipramine (20mg/kg;)
GroupIII : Asparagus racemosus(100mg/kg;)
GroupIV : Asparagus racemosus(200 mg/kg;)

In this model Swiss Albino mice were be divided into 4 groups of six animals each and the test apparatus consists of a transparent rectangular glass jar (25x12x25 cm3) filled to a 15cm depth with water (24 ± 1°C). First group received only saline treatment. In the pre-test session, every animal will be placed individually into the jar for 15mins, 24hrs prior to the 6mins swimming test, in which the duration of immobility is recorded for the last 5mins.1st group receive only saline treatment the 2nd, 3rd, 4th groups receive Oral administration of the graded dose of *Asparagus racemosus*seed extract (100 and 200mg/kg) and Imipramine (20mg/kg p.o.) respectively were be administered one hour prior to final swimming test session. The period between when the mouse was immersed and when no further attempts to escape were made (apart from the movements' necessary to keep its head above the water) will be recorded as the immobility time.

TAILSUSPENSIONMETHOD

GroupI : Control (normal saline-5ml/kg)
GroupII : Imipramine (20mg/kg;)
GroupIII : Asparagus racemosus(100mg/kg;)
GroupIV : Asparagus racemosus(200 mg/kg;)

Thismethodisbasedontheobservationthatamousesuspendedbythetail shows alternating agitationand immobilitythe immobilityisan indicativeofastateofa depression.Swiss Albino mice will be divided into 4 groups of six animals each. Here, the mice were being individually suspended 50 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail 51. Immobility duration was recorded for the last 5 minutes during 6 minutes. Mice were being considered immobile only when they hung passively and were completely motionless. Single administrations of *Asparagus racemosus* seeds extract (100, 200mg/kg) and Imipramine (20mg/kg .) were be given one hr prior to test.[50]

STATISTICAL ANALYSIS

Resultswereanalyzedfor statistical significance byANOVA followed by Dunnet'smultiple comparisontests.Values*P*<0.05&belowwereconsideredsignificant.

RESULTS

Percentage yield after extraction with Methanol as solvent

Plant name : *Asparagus racemosus*

Parts used : Dried seeds

Solvent used: Methanol

Table1:Percentage yield of extract

Weight of plant powder	500 gms
Yield	46.2 gms
Percentage yield	9.24%

Table2: Preliminaryphytochemicalanalysis of *asparagus racemosus*

The revealed results of the preliminary phytochemical analysis of dried seeds extract of *asparagus racemosus* were shown below

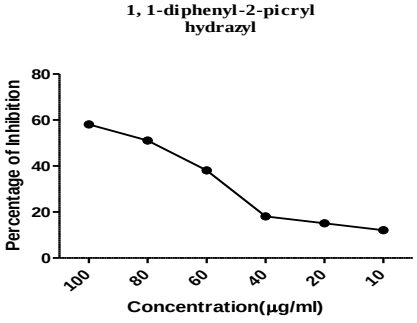
S.no.	Phytochemical Tests	Results
1	Test for carbohydrates	+ve
2	Test for reducing sugars	+ve
3	Test for Monosaccharide's	+ve
4	Test for Hexose sugars	-ve
5	Test for reducing sugars	-ve
6	Test for starch	+ve
7	Test for gums	+ve
8	Test for proteins	-ve
9	Test for steroids	+ve
10	Test for amino acids	-ve
11	Test for flavonoids	+ve
12	Test for Alkaloids	+ve
13	Test for Tannins and Phenolic compound	-ve
14	Test for Glycosides	+ve
15	Test for Saponin glycosides	+ve
16	Test for Coumarin glycosides	+ve

***IN-VTIRO* STUDIES**

Anti- oxidant study[46]

DPPH assay

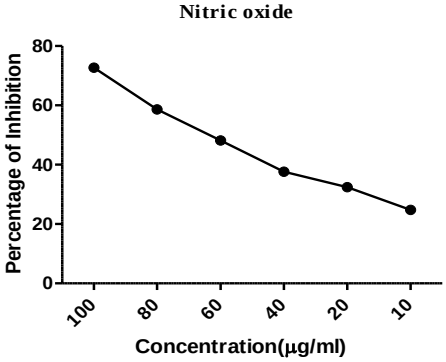
In the DPPH assay, the *in-vitro* antioxidant activity of asparagus racemosus was evaluated for its free radical scavenging activity. Ascorbic acid was taken as a reference standard. The Asparagus racemosus at doses of 100 and 80 µg/ml showed better antioxidant effect by inhibition of free radical scavenging activity and were comparable with standard Ascorbic acid. There was an increase in % inhibition of scavenging effect with the increased concentrations of test compounds (graph 1).



Graph.1-Percentage DPPH scavenging activities ofMethanolicextractof*asparagus racemosus*seeds

Nitric oxide radical scavenging activity[47]

In this assay, the *in-vitro* antioxidant activity of asparagus racemosus was studied for its free radical scavenging activity. Ascorbic acid was taken as a reference standard. The asparagus racemosus at doses of 100 and 80 µg/ml showed better antioxidant effect by inhibition of free radical scavenging activity and were comparable with standard Ascorbic acid. There was an increase in % inhibition of scavenging effect with the increased concentrations of test compounds (graph 2)



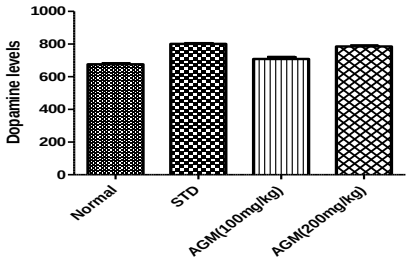
Graph.2Percentagescavenging of nitric oxide radical of Methanolicextractof*asparagus racemosus*

ANTI DEPRESSANT STUDY OF Asparagus racemosus:

In vitro Pharmacological Screening

Estimation of Dopamine level in rat brain by spectrofluorimeter

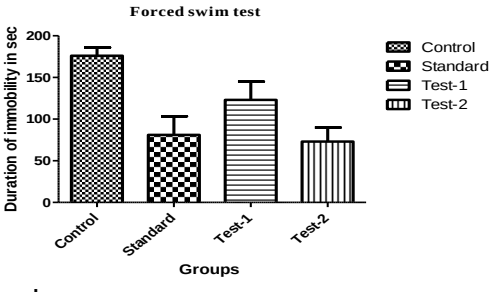
In *in-vitro* study, methanolic extract of asparagus racemosusseeds in the doses of 100mg/kg and 200mg/kg showed increased levels of Dopamine when compared to that of normal. Plant extract at dose of 200 mg/kg showed increased levels of Dopamine, which is nearly equal to that of Standard. This showed that the methanolic seeds extract asparagus racemosusexhibitsAnti depressant activity (Graph-3).



Graph.3 Comparativeprofileof Dopamine Levels of Rat Brainafteracutetreatmentof100mg/kgand200mg/kgofMethanolicextractofasparagus racemosusseeds.

Forced swim test:[48]

Asparagus racemosusat doses of 100 and 200 mg/kg were studied in the forced swim test model of depression in mice. The extract induced a significant ($P<0.05$) reduction in the immobility duration in both dose levels, when compared to the vehicle treated groups. The effect was found to be dosedependant. However, theeffectof Asparagus racemosusat 200mg/kg showed betteractivity than standard drugImipramine (20mg/kg) represented in Graph-4.



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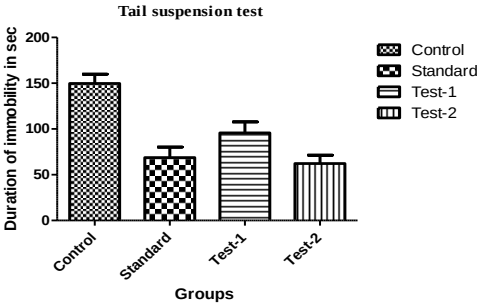
Tail suspension test

The Asparagus racemosus extract was further evaluated in the Tail Suspension Test model of Depression. Asparagus racemosusat doses 100 and 200 mg/kg significantly reduced (P<0.05) the immobility duration in a dose dependent manner when compared to the vehicle treated groups. Imipramine (20mg/kg, po), the standard anti depressant also produced a significant decrease in the immobility time (Graph-5).



Graph.5. Comparative profile of immobility parameter in rat in rat Tail Suspension Test after acute treatment of 100mg/ kg and 200mg/ kg of Methanolicextract of Asparagus racemosus.seeds.

DISCUSSION

Thepurposeofthisstudywastoevaluatetheantidepressantand antioxidant activity of Methanolicextractofasparagus racemosusseedsbyusingbehaviouralanimalmodels and in vitro antioxidant models.Themainfindingofpresentinvestigationsuggeststheantidepressant and antioxidantactivityofMethanolicextractofasparagus racemosusseedsinratforcedswimtest,tailsuspensiontestinmice, Dopamine levels in Rat Brain, DPPH radical scavenging activity and Nitric oxide radical scavenging activity.

Inphytochemicalstudywe observed the presence of flavonoids, alkaloids, steroids glycosides, gums and sugars. The Methanolic extracts of *asparagus racemosus*seed showed the in-vitro antioxidant activity. In DPPH assay and Nitric oxide radical scavenging activity showed the satisfactory results i.e. when the concentration of extract increases, the percentage inhibition of DPPH and Nitric oxide radical scavenging activity also increases and observed the dose dependent action. The antioxidant activity mainly due to the rich of flavonoids in phytochemical analysis, these flavonoids act as antioxidants depend upon their molecular structure. The position of hydroxyl groups &and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities.

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colourstoichometrically depending on the number of electrons taken up. Substances capable of donating electrons/hydrogen atoms are able to convert DPPH (Purple) into their nonradical form 1, 1-diphenyl-2- picrylhydrazine (Yellow), a reaction which can be followed spectrophotometrically. Free radical scavenging activity of the aqueous leaf extract of AC is concentration dependent, as the concentration of the test compounds increases, the radical scavenging activity increased. [52]

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes.. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. From results of Nitric oxide method, it was proved that the *asparagus racemosus* had effective anti oxidant activity. This test extract compete with oxygen to react with NO and thus inhibit the generation of the nitrite and peroxy nitrite anions. [53]

In *in-vitro* study, the methanolic extract of *asparagus racemosus*seeds in the doses of 100mg/kg and 200mg/kg showed increased levels of Dopamine when compared to that of normal. Plant extract at dose of 200 mg/kg showed increased levels of Dopamine, which is nearly equal to that of Standard Imipramine. This showed that the methanolic seeds extract of *asparagus racemosus*exhibitedanti depressant activity.

In *in-vivos*tudy, the methanolic extract of *asparagus racemosus*(100, 200 mg/kg) produced significant antidepressant like effect in both Mice & Rats in both FST & TST; its action was found to be similar to Imipramine. Both the models of depression are widely used to screen new anti-depressant drugs. These tests are sensitive to all major classes of anti-depressant agents.

In FST, Mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavior despair in animals, which is claimed to reproduce a condition similar to human depression. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. [54]

Weobservedthatfollowingadministrationoftestformulationsofasparagusracemosusseedsextractdemonstratedsignificantcomparedtovehicletreatedgroupadosedependantreductionindurationofimmobility and produced significant anti-depressant like effects. The behavioral effects of *asparagus racemosus*are similar to data obtained by other investigators with classical anti-depressant drugs such as Imipramine (or other tricyclic), monoamine oxidase inhibitors and selective serotonin reuptake inhibitors.

The anti-depressant effects of *asparagusracemosus* in FST & TST were more prominent at 200 mg/kg when compared to lower dose of same fraction. The prominent significant antidepressant effects at dose of 200mg/kg could be due to strong and effective concentration of the active constituent.

The swimming and immobility behaviors are sensitive to serotoninergic agents, such as the SSRI's agents. Based on these findings it can be suggested that the *asparagus racemosus* which is able to reduced the immobility time and increase swimming behavior in the Mice exposed to these paradigms can exert its effect through a mechanism similar to that of the SSRI's via serotonin system. [55] More over Imipramine belongs to the class of tricyclic anti-depressant drugs which blocks the reuptake of NE & 5-HT into their respective neurons. Hence *asparagus racemosus* can also mediate its activity through the same mechanism as that of Imipramine.

LIST OF ABBREVIATIONS

TERMS	ABBREVITIONS
5-HT	5-Hydroxy Tryptophan
ANS	Autonomic Nervous System
ANOVA	Analysis of Variance
AGM	*Asparagus racemosus*methanolic extract
AGM-1	100mg/kg *Asparagus racemosus*methanolic

	extract
AGM -2	200mg/kg A*sparagus racemosus*methanolic extract
CNS	Central Nervous System
CPCSEA	Committee for the purpose of control and Supervision of Experiments on Animals
DA	Dopamine
FST	Forced Swim Test
GABA	Gamma Amino Butyric Acid
MAOI	Mono Amine Oxidase Inhibitors
MAO	Mono Amine Oxidase
mTST	Mice Tail Suspension Test
NA	Noradrenaline
OECD	Organization for Economic Co-operation and Development
FST	Forced Swim Test
SSRI	Selective Serotonin Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TST	Tail Suspension Test
WHO	World Health Organization

CONCLUSION

The qualitative analysis of methanolic extract of asparagus racemosusseeds revealed the presence of alkaloids, flavonoids, glycosides, tannins, reducing sugars etc.,themethanolic extract of asparagus racemosusseeds showed in-vitro antioxidant activities i.e. Dpph assay and nitric oxide assay.in in-vitro antidepressant study, the methanolic extract of asparagus racemosusseeds in the doses of 100mg/kg and 200mg/kg showed increased levels of dopamine. The results were nearer to the standard imipramine it indicates the test extract posses antidepressant activity.The investigations of methanolic extract of asparagus racemosusseeds (100mg/kg and 200mg/kg) in both fst&tst models in mice were showed in-vivo antidepressant activity.In this study the results were obtained almost equal to the existed familiar drugs such as ascorbic acid and imipramine. So it is concluded that the methanolic extract of asparagus racemosusseeds should possessed the antioxidant and antidepressant activity.

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