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Preliminary Phytochemical Investigations of *Acacia nilotica* Linn Plant

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Abstract
The plant parts of *Acacia nilotica* linn (AN) has been widely reported to have therapeutic uses arising from its wide spread folkloric and traditional uses. However, very few works has been carried out on the *Acacia* species toward documenting its ethnomedicinal uses and establishing its phytochemical parameters. Establishment of standards of the plant parts will assist in standardization for quality, purity and sample identification. In present study we carried out the characterization of morphological features, determination of physical constant, fluorescence analysis, preliminary phytochemicals screening and TLC profiling of various parts as well as different extracts of AN. This study could be useful to set some diagnostic indices for preparation of monograph, standardization as well as for confirming identity of plant.

Key Words
Acacia nilotica, quality control, ethnomedicine.

Introduction
Acacia nilotica L. is a common, medium sized tree, locally known as 'Babul' or 'Kikar' belongings to the family Mimosaceae. *Acacia* is the most significant genus of family Leguminosae firstly described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide[1, 2]. The plant is considered to be antispasmodic and antidysenteric[3]. Pods and tender leaves are reported to treat diarrhoea[4]. The plant has been shown to exhibit antibacterial[5], antiinflammatory[6], antiplatelet aggregatory activity[7], cestocidal activity[8], antibacterial effects[9], spasmogenic, vasoconstrictor actions[10], antihypertensive, antispasmodic activities[11], inhibitory effect against hepatitis C virus[12], cytotoxic activity[13] and antioxidant activity[14]. Standardization is difficult because herbal drugs are usually mixtures of many constituents and the active principle in most cases is unknown. Therefore the present study was designed to standardize various parts of *Acacia nilotica*.

Materials and Methods
Plant material
The plant parts of *Acacia nilotica* were collected from Medicinal garden of Modern Institute of Pharmaceutical Sciences, Indore and authentified by Head of department of Botany, Holkar Science College, Indore, M.P. for the confirmation of plant identity, The Voucher Specimen was deposited for future reference. The seeds and leaves were shade dried and stems and roots were cut into small fragments and then shade dried. Then dried plant material of seeds, stems and roots was powdered individually by using mixture grinder except leaves which were manually grinded and stored at room temperature for further analysis.

Morphological studies
For morphological observations, various parts of *Acacia nilotica* Linn were examined under magnifying lens and simple microscope[15,16].

Physicochemical studies
The loss on drying, ash values (total ash, acid insoluble ash, water soluble ash), extractive value (petroleum ether, benzene, chloroform, ethanol and water), were determined according to the official methods of Ayurvedic Pharmacopoeia of India[17].

Preliminary phytochemical screening
The dried and powdered plant parts were subjected to maceration with various solvents such as petroleum ether, benzene, chloroform, ethanol and water respectively at ambient temperature for 24 hours and vacuum filtered. The extracts were concentrated to dryness under reduced pressure in a rotary evaporator to yield dried extracts separately. The extracts were subjected to qualitative

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phytochemical investigation and thin layer chromatography for the preliminary identification of the phytoconstituents[18]. TLC plates were first viewed in day light then in UV chamber before keeping in iodine chamber and Rf of all were noted. Different solvent systems were found to be effective to get maximum no. of spots for various extracts.

Fluorescence study

A finely powdered plant parts was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solutions mixed properly and waited for 1-2 minutes. Then the slide was viewed in day light and inside the UV viewer chamber short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded[19].

Results and Discussion

Acacia nilotica is a tree with long grey pods straight or curved constricted between 8-12 seeds. Seeds are extremely hard coated, oblong, 6 -12 mm long, 11-13 mm wide and 3 to 4 cm thick, dark brown to blackish brown (Figure 1). The leaves are bipinnate, pinnate 3-10 pairs, 1- 4 cm long, leaflets 10-20 pairs, and 2-6mm long. Stems are usually dark to black coloured, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum. The branches bear spikes of about 2 cm long. Roots are generally of brown colour in older and whitish in younger regions (Figure 2). Results of loss on drying of various parts of AN are shown in Table No. 1-4. The loss on drying should be reduced in order to prevent microbial contamination. Ash value is a criterion to judge the identity and purity of crude drug. Total ash usually consists of carbonates, phosphates, silicates and silica. Results of ash values are shown in Table No. 1-4. The extractive value (Table No. 5-8) in different solvents is a valuable test to check the quality of drug, and any variation in the chemical constituent. Thus it is an index of the purity of drug. Preliminary qualitative phytochemical screening[20] of different extracts revealed the presence of different phytocompounds (Table No. 9-12). Chromatography is used for the separation and identification of various phytochemicals present in the extracts. In the present study TLC has been conducted for the separation of various components and *Rf* values of developed spots of different extracts were calculated with color intensity (Table 13-16).The fluorescence characteristic of any powdered drug is very distinctive and helpful in distinguishing features for the determination of the drug content (Table 17-20)[20].

Conclusion

Present study may be useful to supplement information in respect of identification authentication, adulteration and standardization of various parts of *A. nilotica*.

Acknowledgement

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Table 1: Loss on drying (LOD) and ash values of powdered of *A. nilotica* seeds.

Parameters	**Average Values (%)**
Loss on drying	11.08
Total ash	4.01
Acid insoluble ash	1.61
Water soluble ash	1.46

Table 2: Loss on drying (LOD) and ash values of powdered of *A. nilotica* leaves.

Parameters	**Average Values (%)**
Loss on drying	13.10
Total ash	6.23
Acid insoluble ash	1.34
Water soluble ash	2.31

Table 3: Loss on drying (LOD) and ash values of powdered of *A. nilotica* stem.

Parameters	Average Values (%)
Loss on drying	12.02
Total ash	5.04
Acid insoluble ash	1.01
Water soluble ash	2.27

Table 4: Loss on drying (LOD) and ash values of powdered of *A. nilotica* roots.

Parameters	Average Values (%)
Loss on drying	10.03
Total ash	4.17
Acid insoluble ash	1.91
Water soluble ash	1.71

Table 5: Percent extractives and colors of successive extracts of *A. nilotica* seeds.

Solvent	% Extractive	Colors of extracts
Pet. Ether	3.21	Pale yellow
Benzene	4.36	Pale yellow
Chloroform	5.03	Yellow
Ethanol	13.47	Dark yellow
Water	15.38	Yellowish orange

Table 6: Percent extractives and colors of successive extracts of *A. nilotica* leaves.

Solvent	% Extractive	Colors of extracts
Pet. Ether	2.71	Light Green
Benzene	3.78	Yellowish Brown
Chloroform	6.23	Dark Green
Ethanol	14.17	Dark Green
Water	17.28	Dark Brown

Table 7: Percent extractives and colors of successive extracts of *A. nilotica* stem.

Solvent	% Extractive	Colors of extracts
Pet. Ether	1.56	Light Yellowish Green
Benzene	2.23	Dark Yellowish Green
Chloroform	5.22	Dark Yellow
Ethanol	10.43	Dull Brown
Water	12.34	Yellowish Brown

Table 8: Percent extractives and colors of successive extracts of *A. nilotica* root.

Solvent	% Extractive	Colors of extracts
Pet. Ether	1.11	Buff colour
Benzene	2.33	Buff Colour
Chloroform	3.10	Light orange
Ethanol	9.56	Reddish Brown
Water	11.28	Yellowish Brown

Table 9: Results of phytochemical screenings of successive extracts of *Acacia nilotica* seeds.

Chemical Constituents	Pet. Ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	-ve	-ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	+ve	+ve
Glycosides	-ve	-ve	-ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	-ve
Flavonoids	-ve	-ve	-ve	+ve	+ve
Saponins	-ve	-ve	-ve	+ve	+ve
Fixed oils and fats	+ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	+ve	+ve
Proteins and amino acids	-ve	-ve	-ve	-ve	+ve
Terpenoids	+ve	+ve	-ve	-ve	-ve

+ = Present, - = Absent.

Table 10: Results of phytochemical screenings of successive extracts of *Acacia nilotica* **leaves.**

Chemical Constituents	Pet. Ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	-ve	-ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	+ve	+ve
Glycosides	-ve	-ve	-ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	+ve	+ve	+ve
Saponins	-ve	-ve	-ve	+ve	+ve
Fixed oils and fats	-ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	+ve	+ve
Proteins and amino acids	-ve	-ve	-ve	+ve	+ve
Terpenoids	+ve	+ve	-ve	-ve	-ve

+ = Present, - = Absent.

Table 11: Results of phytochemical screenings of successive extracts of *Acacia nilotica* stem.

Chemical Constituents	Pet. Ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	+ve	+ve	+ve	+ve	-ve
Carbohydrates	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	-ve
Flavonoids	-ve	-ve	-ve	+ve	+ve
Saponins	-ve	-ve	-ve	+ve	+ve
Fixed oils and fats	-ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	+ve	+ve
Proteins and amino acids	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	-ve	-ve	-ve

Table 12: Results of phytochemical screenings of successive extracts of *Acacia nilotica* roots.

Chemical Constituents	Pet. Ether extract	Benzene extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	-ve	-ve	-ve	-ve	-ve
Carbohydrates	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve	-ve
Steroids	+ve	+ve	+ve	+ve	-ve
Flavonoids	-ve	-ve	-ve	+ve	+ve
Saponins	-ve	-ve	-ve	+ve	+ve
Fixed oils and fats	+ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	+ve	+ve
Proteins and amino acids	-ve	-ve	-ve	-ve	-ve
Terpenoids	-ve	-ve	-ve	-ve	-ve

+ = Present, - = Absent.

Table 13: Observations of thin layer chromatographic studies of *A. nilotica* seeds.

Extracts	**Mobile phase**	**No. of spots**	**Rf values**	**Color**	**Intensity**
Petroleum Ether	Benzene: Chloroform (1:1)	1	0.20 (UV)	Y	+++
Benzene	Chloroform: ethanol (9.5:0.5)	4	0.55 (V) 0.46 (V) 0.75 (UV) 0.15 (UV)	Y Y Y Y	+++ ++ ++ +
Chloroform	Chloroform: ethanol (9.5:0.5)	4	0.46 (V) 0.62 (V) 0.66 (UV) 0.40 (UV)	.Y Y Y Y	+++ ++ +++ +++
Ethanol	Chloroform: ethanol (8:2)	3	0.86 (UV) 0.52 (UV) 0.15 (UV)	Y Y Y	++ ++ +
Water	1 Butanol: Acetic acid: Water (4:1.1:4.9)	1	0.75 (UV)	Y	+++

+++ = Most intense, ++ = moderately intense, + = Least intense, Y=Yellow, (V) = Visible, (UV) = Ultraviolet 365 nm.

Table 14: Observations of thin layer chromatographic studies of *A. nilotica* leaves.

Extracts	**Mobile phase**	**No. of spots**	**Rf values**	**Color**	**Intensity**
Petroleum Ether	Benzene: Chloroform (1:1)	6	0.10 (V, UV) 0.15 (V, UV) 0.96 (V, UV) 0.16 (I) 0.33 (I) 0.81 (I)	G Gr Y Y Y Y	+++ +++ ++ ++ + +++
Benzene	Chloroform: ethanol (9.5:0.5)	2	0.92 (V) 0.92 (UV) 0.10 (V) 0.10 (UV)	G Br Y Y	+++ + ++ +++
Chloroform	Chloroform: ethanol (9.5:0.5)	6	0.60 (V) 0.70 (V) 0.80 (V) 0.84 (UV) 0.50 (I) 0.80 (I)	Gr Gr G O Y Y	+ ++ +++ +++ + +
Ethanol	Chloroform: ethanol (8:2)	4	0.20 (V) 0. 70 (V) 0.76 (UV) 0.62 (I)	Br LG O Gr	+++ +++ +++ +++
Water	Butanol: Acetic acid: Water (4:1.1:4.9)	6	0.20 (V) 0.40 (V) 0.46 (V) 0.54 (UV) 0.30 (I) 0.48 (I)	Y Y Y YF G Y	+++ ++ + +++ ++ ++

+++ = Most intense, ++ = moderately intense, + = Least intense, Y=Yellow, G=Green, LG= Light Green, Gr = Grey, Br= Brown, O=Orange, YF= Yellow Fluorescence, (V) = Visible, (UV) = Ultraviolet 365 nm.

Table 15: Observations of thin layer chromatographic studies of *A. nilotica* stem.

Extracts	Mobile phase	No. of spots	Rf values	Color	Intensity
Petroleum Ether	Benzene: Chloroform (1:1)	5	0.05 (UV) 0.06 (UV) 0.96 (UV) 0.13 (I) 0.25 (I)	O Y Y Y Y	+++ ++ +++ + +
Benzene	Chloroform: ethanol (9.5:0.5)	7	0.78 (V) 0.96 (V) 0.80 (UV) 0.90 (UV) 0.98 (UV) 0.30 (I) 0.50 (I)	G Y Y O Y Y Y	+++ ++ +++ +++ +++ ++ ++
Chloroform	Chloroform: ethanol (9.5:0.5)	6	0.75 (V) 0.95 (V) 0.67 (UV) 0.87 (UV) 0.77 (I) 0.92 (I)	Y G Y Gr Gr Br	+++ +++ +++ +++ ++ ++
Ethanol	Chloroform: ethanol (8:2)	3	0.204 (UV) 0.714 (UV) 0.938 (UV) 0.102 (I) 0.408 (I)	Y Y O Br Br	++ + +++ ++ +
Water	1 Butanol: Acetic acid: Water (4:1.1:4.9)	2	0.71 (V) 0.64 (I)	Br Gr	++ ++

+++ = Most intense, ++ = moderately intense, + = Least intense, Y=Yellow, O=Orange, G= Green, Gr= Grey, Br=Brown (V) = Visible, (UV)= Ultraviolet 365 nm.

Table 16 : Observations of thin layer chromatographic studies of *A. nilotica* root.

Extracts	Mobile phase	No. of spots	Rf values	Color	Intensity
Petroleum Ether	Benzene: Chloroform (1:1)	4	0.10 (UV) 0.16 (UV, I) 0.25 (I) 0.81 (I)	Y Y Y Br	+++ +++ ++ ++
Benzene	Chloroform: ethanol (9.5:0.5)	1	0.96 (UV)	Y	++
Chloroform	Chloroform: ethanol (9.5:0.5)	1	0.55 (UV)	O	+++
Ethanol	Chloroform: ethanol (8:2)	2	0.40 (UV) 0.68 (I)	Y Y	+ ++
Water	1 Butanol: Acetic acid: Water (4:1.1:4.9)	2	0.60 (UV) 0.58 (I)	Y Y	++ ++

+++ = Most intense, ++ = Moderately intense, + = Least intense, Y=Yellow, Br= Brown, O=Orange, (V) = Visible, (UV)= Ultraviolet 365 nm.

Table 17: Fluorescence analysis of powdered *A. nilotica* seeds.

S. No.	**Powdered drug + Reagent**	**Observation**		
		Seed		
		Visible/Day light	**UV (Long)**	**UV (Short)**
1	Powder + 1 M NaOH	Dark Brown	Yellowish brown	Black
2	Powder + CH_3COOH	Brown and outer side yellowish brown	Yellow at center and brown at edges.	Yellow at center and brown at edges.
3	Powder + 1 M HCl	Light brown at center and brown at edges.	Black	Yellowish Black
4	Powder + 5% I_2	Dark Brown	Yellowish-Green	Yellowish Black
5	Powder + 5% $FeCl_3$	Black	Black	Black
6	Powder + Methanol	Light yellow at center and brown at edges.	Light yellow at center and brown at edges.	Fluorescent yellow at center and brown at edges.
7	Powder + 1 M H_2SO_4	Dark Brown	Black	Black with fluorescent yellow
8	Powder + Conc. HNO_3	Orangish Yellow	Dark yellow	Orange
9	Powder + $K_2Cr_2O_7$	Dark yellow at center and brown at edges.	Yellowish Black	Black with fluorescent yellow
10	Powder + 1 N NaOH	Brownish-black	Yellowish Black	Yellowish Black
11	Powder + 1 N NaOH (Ethanolic)	Black	Black	Black
12	Powder + 1 N HCl	Brown	Yellow at center and brown at edges.	Yellow at center and brown at edges.
13	Powder + 1 N H_2SO_4	Light brown	Yellowish-brown	Yellowish-brown
14	Powder + dil. HNO_3	Orange Brown	Yellowish-brown	Yellowish-brown
15	Powder + 25% NH_3	Yellowish-brown	Yellowish-brown	Black
16	Powder + dil. NH_3	Yellowish-brown	Fluorescent yellow at center and brown at edges.	Fluorescent yellow at center and brown at edges.
17	Powder + 50% HNO_3	Orangish-yellow	Yellowish-brown	Yellowish-Orange
18	Powder + HNO_3 + 25% NH_3	Orangish-yellow	Orangish-yellow	Orangish-yellow

Table 18: Fluorescence analysis of powdered *A. nilotica* leaves.

S. No.	**Powdered drug + Reagent**	**Observation**		
		Leaves		
		Visible/Day light	**UV (Long)**	**UV (Short)**
1	Powder + 1 M NaOH	Yellow at center Edges-blue	Blue	Black
2	Powder + CH_3COOH	Grey	Yellow at edges and blackish-grey at centre	Brown at edges and fluorescent yellow at center
3	Powder + 1 M HCl	Green	Grey	Yellowish-black
4	Powder + 5% I_2	Edges-yellow Centre-black	Edges-yellow Centre-black	Yellowish-black
5	Powder + 5% $FeCl_3$	Black	Black	Black
6	Powder + Methanol	Light-green	Grey	Fluorescent yellow
7	Powder + 1 M H_2SO_4	Centre-yellow Edges-green	Centre-yellow Outer-grey	Black with fluorescent yellow in centre
8	Powder + Conc. HNO_3	Orangish Yellow	Orange	Orange
9	Powder + $K_2Cr_2O_7$	Outer- yellow Inner-black Centre- orange	Yellow at Edges, orange at center.	Black with yellow
10	Powder + 1 N NaOH	Yellowish-brown	Outer-dark yellow Edges-black	Yellowish Black
11	Powder + 1 N NaOH (Ethanolic)	Yellowish-brown	Black	Blue
12	Powder + 1 N HCl	Green	Grayish-black	Yellow at center and brown at edges.
13	Powder + 1 N H_2SO_4	Yellowish-green	Yellowish-green	Yellowish-black
14	Powder + dil. HNO_3	Orange	Yellowish-brown	Yellowish-brown
15	Powder + 25% NH_3	Yellowish-black	Yellowish-black	Black
16	Powder + dil. NH_3	Dark Yellowish-green	Dark Yellowish-green	Black
17	Powder + 50% HNO_3	Orange	Yellowish-brown	Orangish-brown
18	Powder + HNO_3 + 25% NH_3	Yellowish-Orange	Yellow	Orange

Table No. 19: Fluorescence analysis of powdered *A. nilotica* stem.

S. No.	**Powdered drug + Reagent**	**Observation**		
		Stem		
		Visible/Day light	**UV (Long)**	**UV (Short)**
1	Powder + 1 M NaOH	Dark yellow	Light Yellow	Fluorescent yellow
2	Powder + CH_3COOH	Light Yellow	Fluorescent yellow	Fluorescent yellow
3	Powder + 1 M HCl	Blue	Black	Black
4	Powder + 5% I_2	Dark yellow	Yellowish- Green	Yellow
5	Powder + 5% $FeCl_3$	Black	Black	Black
6	Powder + Methanol	Light yellow	Dark Brown	Brownish-black
7	Powder + 1 M H_2SO_4	Orange	Yellowish-brown	Yellowish-Black
8	Powder + Conc. HNO_3	Light yellow	Light yellow	Light yellow
9	Powder + $K_2Cr_2O_7$	Brownish-orange	Light yellowish brown	Brownish-orange
10	Powder + 1 N NaOH	Yellowish-brown	Yellowish-brown	Yellowish Black
11	Powder + 1 N NaOH	Black	Brown	Brown
12	Powder + 1 N HCl	Bluish-purple	Bluish-brown	Black
13	Powder + 1 N H_2SO_4	Orange Brown	Yellow	Yellowish-brown
14	Powder + dil. HNO_3	Light yellow	Light yellow	Dark yellow
15	Powder + 25% NH_3	Yellow	Light yellow	Light yellow
16	Powder + dil. NH_3	Yellowish-brown	Brownish-yellow	Dark Brown
17	Powder + 50% HNO_3	Light yellow	Light yellow	Light yellow
18	Powder + HNO_3 + NH_3	Light yellow	Light yellow	Dark yellow

Table 20: Fluorescence analysis of powdered *A. nilotica* roots.

S. No.	**Powdered drug + Reagent**	**Observation**		
		Root		
		Visible/Day light	**UV (Long)**	**UV (Short)**
1	Powder + 1 M NaOH	Dark yellow	Light yellow	Fluorescent yellow
2	Powder + CH_3COOH	Light yellow	Light yellow	Dark yellow
3	Powder + 1 M HCl	Blue	Black	Black
4	Powder + 5% I_2	Dark yellow	Yellow	Yellow
5	Powder + 5% $FeCl_3$	Black	Black	Black
6	Powder + Methanol	Light-yellow	Dark brown	Brownish-black
7	Powder + 1 M H_2SO_4	Orange	Yellowish-brown	Yellowish-brown
8	Powder + Conc. HNO_3	Light-yellow	Light-yellow	Light-yellow
9	Powder + $K_2Cr_2O_7$	Brownish-orange	Light yellowish brown	Brownish-orange
10	Powder + 1 N NaOH	Yellowish-brown	Yellowish-brown	Yellowish Black
11	Powder + 1 N NaOH	Black	Brown	Brown
12	Powder + 1 N HCl	Bluish-purple	Blackish-brown	Black
13	Powder + 1 N H_2SO_4	Orangish-brown	Yellow	Yellowish-brown
14	Powder + dil. HNO_3	Light-yellow	Light-yellow	Dark-yellow
15	Powder + 25% NH_3	Yellow	Light-yellow	Light-yellow
16	Powder + dil. NH_3	Yellowish-brown	Yellow	Black
17	Powder + 50% HNO_3	Light-yellow	Light-yellow	Dark-yellow
18	Powder + HNO_3 + NH_3	Yellow	Yellowish-brown	Fluorescent yellow

Figure 1: Seed of *Acacia nilotica Linn*

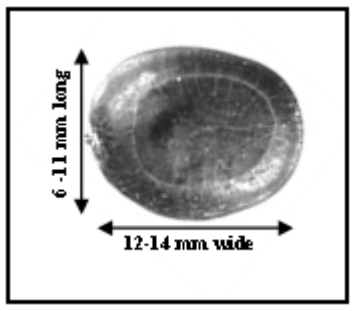

Figure 2: Plant of *Acacia nilotica Linn*

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