Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (3):** 271-279 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

ResearchArticle

OPEN ACCESS

Phytochemical Screening and Antimicrobial Activity of Medicinal Plants (*Eclipta prostrata* L. and *Sphaeranthus indicus* L.)

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ABSTRACT

Preliminary phytochemical and antibacterial screening was conducted using methanol ethanol and chloroform extracts of Eclipta prostrata L. and Sphaeranthus indicus L.Preliminary phytochemical screening was done on Methanol extract, Ethanol extract and chloroform extract in which the methanolic extract revealed maximum number of phyto-constituents. This study revealed the presence of Alkaloids, flavonoids, Tannins, Terpenoids, Steroids and Glycosides.In antibacterial screening both the plants exhibited significant activity against Shigella boydii, Escherichia coli, Klebsiella pnuemoniae, Pseudomonos Sps, Salmonella paratyphi A.The secondary metabolites are responsible for the medicinal activity of studied plants. This study concluded that E.prostrata L. and Sphaeranthus indicus L. have sufficient antibacterial activity due to the presence of secondary metabolites.

Key words: *Phyto-constituents, antibacterial activity, Secondary metabolites, Asteraceae*

INTRODUCTION

Medicinal plants are the only important natural source to the development of drugs without any adverse effects. In which the secondary metabolites are responsible for the activity against harmful pathogens. The first step towards this goal is the biological and phytochemical screening of plant extracts and:or extracts from traditional preparations used in popular medicine[1,2]. Successful strategies for investigation of these preparations involve the selection of test crude extracts based on a combination of ethnopharmacology and daily healer's practices.The presence of secondary metabolites was very important for the medicinal activity. It was confirmed by preliminary phytochemical analysis using harborne method[3].There is a constant and crucial need to innovate novel antimicrobial compounds with diverse chemical structures and unique mechanisms of action because there has been a disquieting increase in the incidence of new and re-emerging infectious diseases. Another massive concern is the development of resistance to the antibiotics in contemporary medical use. The present study focused to reveal the antibacterial activity of Asteraceae members *Eclipta prostrata* L. and *Sphaeranthus indicus* L. In this paper we report the antibacterial activity of studied medicinal plants against 5 bacterial strains.

MATERIALS AND METHODS

Details of the study plant

Eclipta prostrata L. and *Sphaeranthus indicus* L. was collected from in and around places of Musiri. Leaves, root and stem were excised from mother plant and used as explants for further experiments.

Preliminary phytochemical Analysis[3]:

The *Eclipta prostrata* L. and *Sphaeranthus indicus* L. plant parts like leaves, stem, and root were collected and dried under shade condition, ground to powder using an electric blender and dissolved separately in 100 ml of solvent. This solution was kept under room temperature for seven days to allow the extraction of compounds from leaf, stem flowers and root. The solution of each sample was stirred after every 24 hours using sterile glass rods. After seven days, the solution was filtered through what man No-1filter paper. The solvent was evaporated and sticky substance obtained that was stored in the refrigerator and suspended in 10% (DMSO) Di-methyl Sulfoxide prior to use.

Chemical test were carried out with both the plants extracts and on the powder specimens using standard procedure to identify the constitutions as described by Harborne[3], the specific procedure involved for the evaluations of a particular group of chemical is mention below.

Tannins

1 ml of water and 1-2 drops of ferric chloride solution were added in 0.5ml of extracted solution. Blue color was observed for tannis and green black for methanolic tannin.

Saponins (Foam test)

Small amount of extract was shaken well with little quantity of water. If foam produced persist for ten minutes if the presence of saponins.

Flavonoids (Alkaline Reagent test)

Extractions were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which because colors on addition of acid, Indicates the presence of flavonoids.

Steroids

2 ml of acetic anhydride was added to 0.5g extract of each sample with 2 ml H_2SO_4. The color changed from violet or blue or Green in some samples indicating the presence of steroids.

Terpenoides (salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to forum a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids.

Cardiac glycosides (Keller-Killani test)

5 ml of each extract was treated with 2 ml of glacial acetic containing drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring of inter face indicates a deoxysugar characteristics of carotenoides. A violet ring may appear below ring while in the acetic layer, a greenish ring may from just gradually throughout thin layer.

Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloids solution produces while yellowish precipitated a few drops of Mayer's reagents are added.

Anthraquinones

Born forager's test was used for detecting the presence of anthroquinone. In this case 0.5g of planet extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red, or violet coloration in the ammonia phase indicated the presence of anthraquinone.

Anti bacterial assay

Plant Material

The *in vivo* plant parts were collected from natural habitats in and around places of Musiri. The plant parts like *in vivo* leaves, stem, and roots were shade dried at ambient temperature (31°C) and the dried samples were crushed into fine powder using an electric Mixer.

Extract Preparation

The powdered plant materials were soaked separately in methanol in a Soxhlet apparatus for 72 hr at 31°C until complete exhaustion of the material. The mixture was stirred at every 24 hr using a sterile glass rod. At the end of 72 hr, the extract was passed through Whatman.No.1 filter paper and the filtrates were concentrated in vacuum rotary evaporator at 60°C in order to reduce the volume. The paste like extracts were stored in labeled screw capped bottles and kept in refrigerator at 4°C. The extract was reconstituted using minimal amounts of methanol prior to use.

Microorganisms

The pathogenic bacterial species were collected from the Department of Microbiology, K.A.P Vishwanathan Govt. Medical College, Tiruchirappalli, Tamil Nadu. Bacterial strains consisted of *Shigella boydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp.*and *Salmonella paratyphi* A. The bacterial strains were maintained in Nutrient Agar (Hi Media Laboratories Pvt. Ltd., Mumbai). The strains were subcultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5°C for further analysis.

Evaluation of Antibacterial activity

The antibacterial activity of the methanolic extracts of various parts and *in vitro* grown plant of *Eclipta prostrata*L. and *Sphaeranthus indicus* L. was evaluated through disc-diffusion method.

Disc-diffusion Test[4]

The disc-diffusion method provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnating small circular discs of standard filter paper with the given amount of a chosen concentration of substance. The discs are placed on plates if culture medium previously spread with a bacterial inoculum to be tested. After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the discs into the surrounding medium.Sterilised disc of (Himedia, Mumbai) were used in the study. These discs were impregnated with each plant extract for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37° C for 24 hrs. The zone of inhibition around each disc was measured and the diameter recorded. Gentamycin (10 mcg/disc) was used as the reference. A negative control was prepared using only the solvent used for extraction and kept for comparison. The tests were repeated 4 times to ensure reliability of the result.

RESULTS AND DISCUSSION

Preliminary phyto-chemical analysis of *Eclipta prostrata* L. and *Sphaeranthus indicus* L.:

Herbal medicine signifies one of the most important fields of traditional medicine in all over the world. To encourage the appropriate use of herbal medicine used to regulate their potentialityas sources for new drugs. Preliminary phytochemical analysis was carried out to understand the presence of various chemical constituents in *E.prostrata* L. and *S.indicus* L. Methanolic, Ethanolic and Chloroform derived crude extracts of different parts of the proposed plants were analyzed. From the qualitative analysis, it was observed that both the plantscontain Alkaloids, Tannins, Cardiac glycosides, Terpenoids, Flavanoids and Steroids (Table 1-6, Figure 1-6). Similarly the phytochemical screening and quantitative estimation was reported by[5,6] of the following plants like *Galium aparine, Rumex dentatus, Avena fatua, Lathyrus aphaca, Phalaris* minor the crude extract of the plant seeds studies showed that the seeds were rich in alkaloids, flavonoids, tannins, cardiac glycosides and saponins. The extracts have medicinal activity as well as exhibiting physiological activity. The phyto chemical screening shows the presence of medicinally active compounds in *E.prostrata* L. and *Sphaeranthus indicus* L.

Fig. 1: Preliminary phytochemical screening of *Eclipta prostrata* L. leaf explant

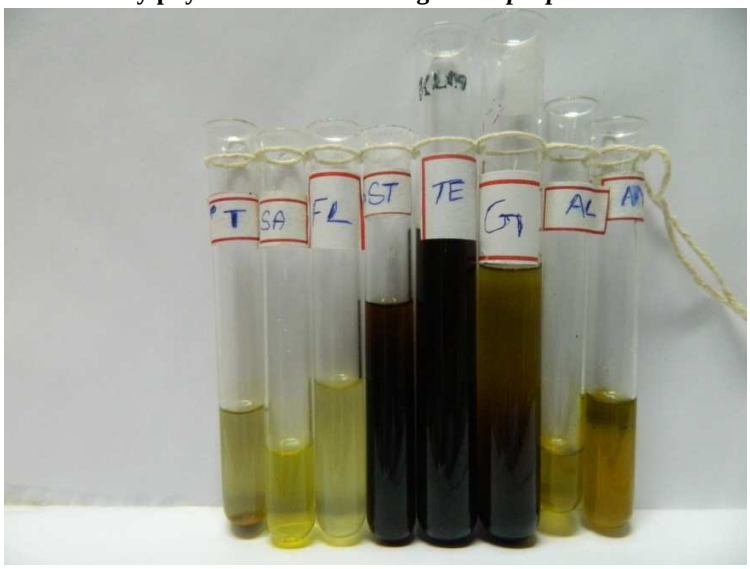


Table 1: Preliminary phytochemical analysis of*E.prostrata* L. leaf explant using various solvents

S. No.	Test	Methanol	Ethanol	Chlorofom
1	Tannins	-	+	-
2	Saponins	-	-	-
3	Flavanoids	+	-	+
4	Steroids	+	-	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	+	+	+
8	Anthraquinones	-	-	-

Fig. 2: Preliminary phytochemical screening of *Eclipta prostrata* L. stem explant

Table 2: Preliminary phytochemical analysis of*E.prostrata* L. stem explant using various solvents

S.No	Test	Methanol	Ethanol	Chloroform
1	Tannins	-	+	-
2	Saponins	+	_	+
3	Flavanoids	+	-	-
4	Steroids	+	+	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	-	-
7	Alkaloids	-	-	-
8	Anthraquinones	+	+	+

Fig. 3: Preliminary phytochemical screening of *Eclipta prostrata* L. root explant

Table 3: Preliminary phytochemical analysis of*E.prostrata* L. root explant using various solvents

S.No	Test	Methanol	Ethanol	Chloroform
1	Tannins	-	-	-
2	Saponins	-	+	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	-	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	-	-	-
8	Anthraquinones	-	-	-

Fig. 4: Preliminary phytochemical screening of *Sphaeranthus indicus* L. Leaf explant

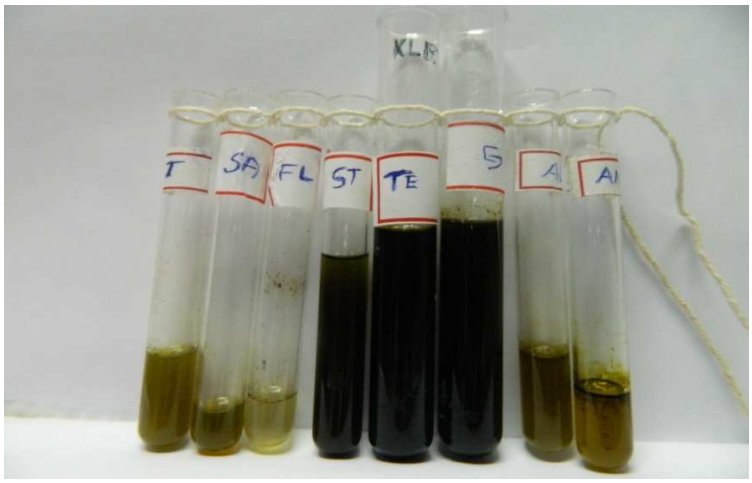


Table 4: Preliminary phytochemical analysis of*S.indicus* L. leaf explant using various solvents

S. No.	Test	Methanol	Ethanol	Chloroform
1	Tannins	-	+	-
2	Saponins	-	-	-
3	Flavanoids	+	+	+
4	Steroids	+	-	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	+	+	+
8	Anthraquinones	-	-	-

Fig. 5: Preliminary phytochemical screening of *Sphaeranthus indicus* L. stem explant

Table 5: Preliminary phytochemical analysis of*S.indicus* L. stem explant using various solvents

S.No.	Test	Methanol	Ethanol	Choloroform
1	Tannins	-	+	-
2	Saponins	+	_	+
3	Flavanoids	+	+	-
4	Steroids	+	+	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	-	-
7	Alkaloids	-	+	-
8	Anthraquinones	-	-	-

Fig. 6: Preliminary phytochemical screening of *Sphaeranthus indicus* L. root explant

Table 6: Preliminary phytochemical analysis of*S.indicus* L. root explant using various solvents

S.No	Test	Methanol	Ethanol	Chloroform
1	Tannins	-	+	-
2	Saponins	-	-	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	-	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	-	+	-
8	Anthraquinonus	-	-	-

Antibacterial Activity:

Disc Diffusion Method

The methanolic extracts taken for antibacterial study because methanolic extracts yield more phyto constituents than others in preliminary phytochemical analysis. The results showed that different parts of the study plantsexhibited activity against the pathogenic bacteria used in the experiment. The inhibitory action was observed in terms of diameter of inhibition zone formed around each disc caused by the

diffusion of anti-bacterial substances from the paper disc into the surrounding medium a close examination of the results showed (Table 7 & 8, Figure 7 & 8) that the extent of antibacterial activity of methanolic extract against each of the bacterial strains follows a rank of order. In other words, antibacterial activity was higher in *in vivo* root extract and very low in *in vivo* stem extract. The diameter of inhibition zone for each of the sample against every tested microorganisms was found to be either less than or equal to that of the standard antibiotic disc (Gentamycin, 10 mcg/disc) used in the assay. Seenivasan[3]screened 21 essential oils. Cinnamon, clove, geranium, lemon, lime, orange and rosemary oils exhibited significant inhibitory effect. In general, *B.subtilis* was more susceptible. On the other hand *K. pneumoniae* exhibited low degree of sensitivity. Zaidan[8]carried out antibacterial screening in the leaves of *Andrographis paniculata*, *Vitex negundo*, *Morinda citrifolia*, *Piper sarmentosum*, and *Centella asiatica* against five strains of bacterial species, Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*, using standard protocol of Disc Diffusion Method (DDM). The antibacterial activities were assessed by the presence or absence of inhibition zones and MIC values. *Morinda citrifolia*, *Piper sarmentosum* and *Centella asiatica* methanol extract and *Andrographis paniculata* (water extract) have potential antibacterial activities to both gram positive *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA). None of the five plant extracts tested showed antibacterial activities to gram negative *E. coli* and *Klebsiella pneumoniae*, except for *A. paniculata* and *Pseudomonas sarmentosum* which showed activity towards *Pseudomonas aeruginosa*. *Andrographis paniculata* being the most potent at MIC of 2 µg/disc. Doughari[9]carried out antimicrobial studies in the leaf extracts of *Senna obtusifolia* (L) against both clinical and laboratory isolates of both bacteria and fungi using the disc diffusion method.

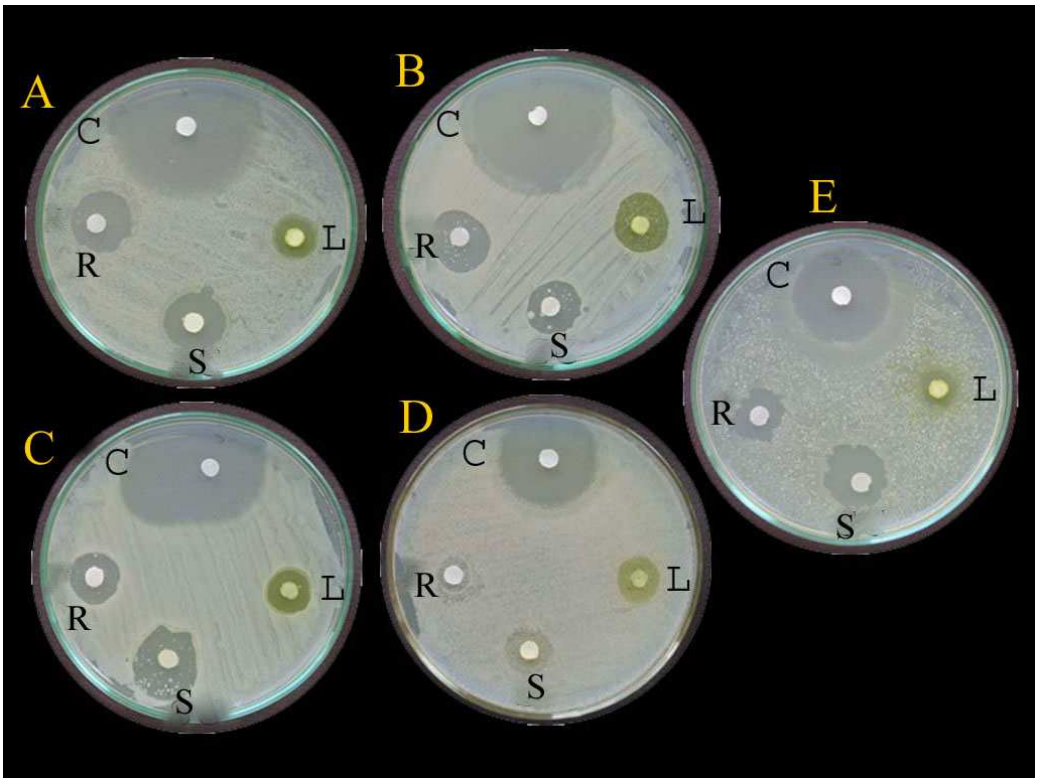
Table 7: Zone of inhibition of produced by *E. prostrata* L.

S. No.	Number of organisms	Methanol			Ethanol			Chloroform		
		L mm	S mm	R mm	L mm	S mm	R mm	L mm	S mm	R mm
1.	*Shigella boydii*	3.9	3.4	3.1	3.5	3.2	2.9	3.2	2.6	2.1
2.	*E.coli*	3.2	2.9	3.0	1.5	3.0	2.0	3.0	3.0	2.6
3.	*Klebsiella pneumoniae*	3.0	3.1	4.0	3.1	1.1	2.9	3.1	3.1	2.3
4.	*Pseudomonas Sp*	1.9	1.2	1.7	0.6	0.7	0.5	3.7	3.0	2.5
5.	*Salmonella Paratyphi* A	3.2	2.1	1.4	1.7	1.5	1.9	3.0	2.0	2.0

Table 8: Zone of inhibition of produced by *S.indicus* L.

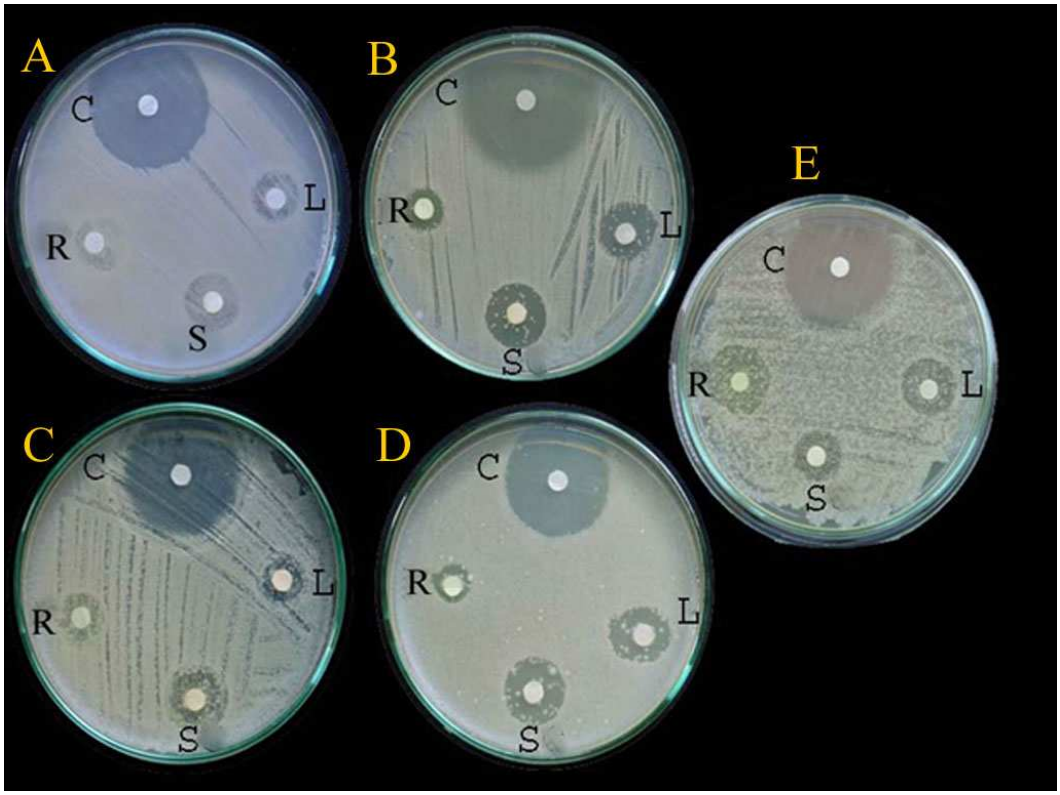
S. No.	Number of organisms	Methanol			Ethanol			Chloroform		
		L mm	S mm	R mm	L mm	S mm	R mm	L mm	S mm	R mm
1.	*Shigella boydii*	3.4	1.9	3.1	3.2	2.5	2.9	3.9	2.6	3.1
2.	*E.coli*	3.2	2.9	3.0	3.5	1.5	2.0	3.0	3.0	2.6
3.	*Klebsiella pneumoniae*	4.0	3.1	3.0	3.1	1.1	2.9	4.0	3.1	2.9
4.	*Pseudomonas Sp.*	1.9	1.2	1.7	0.6	0.7	0.5	3.9	3.0	2.5
5.	*Salmonella Paratyphi* A	3.2	2.1	1.2	1.7	1.5	1.9	3.0	2.0	2.3

Fig. 7: Antibacterial activity of *Eclipta prostrata* L. (Methanolic extract)



A-S*higella boydii;* **B-** *E.coli;* **C-** *Klebsiella pneumonia;* **D-** *Pseudomonas Sp.;* **E-** *Salmonella Paratyphi* A; C-Control; L- Leaf extract; S – Stem extract; R – Root extract.

Fig. 8: Antibacterial activity of *Sphaeranthus indicus* L. (Methanolic extract)



A-S*higella boydii;* **B-** *E.coli;* **C-** *Klebsiella pneumonia;* **D-** *Pseudomonas Sp.;* **E-** *Salmonella Paratyphi* A; C-Control; L- Leaf extract; S – Stem extract; R – Root extract.

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