# Investigation of 'African tulip tree-calyx water' as a novel source water, food and medicine.

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Abstract

It's very interesting to observe the birds sucking the water from flower bud of a particular plant. Actually these observations lead us for Pharmacognostical and Physiochemical standardization of Water Calyx Fluid in the African tulip tree i.e. *Spathodea campanulata* P. Beauv. The healthy buds were selected and grouped into small, medium and large sizes based on the size cum weight of intact flower buds. For physicochemical study, calyx fluid from all the bud sizes were collected freshly, mixed and analyzed qualitatively and quantitatively for pharmacognostical and physiochemical standardization with well defined methods. Calyx fluid of S. *campanulata* shows high in the concentration of reducing sugar, phenol, protein and amino acids which indicate its absolute necessity for the floral development. The concentrations of all the analyzed biomolecules were found to be decreasing with bud maturity. The contents of water calyx fluid are obligatory for the development of floral whorls. It can be said that, there is good potential in high nutritive calyx fluid can be used a nutritional supplement and novel food source. Presence of phytohormones like IAA, IBA and Siderophore in the calyx fluid in bud maturity suggests that the calyx fluid has a vital role in the exponential development of corolla. Presence of antimicrobial activity indicates the role of calyx fluid in immunological defense mechanism of *S. campanulata*.

Key Words: Floral development, Phytohormones, Calyx fluid, Antimicrobial activity

**Introduction:**

It is very interesting if flower water is use for consumption by birds, especially when there is low or no precipitation (Janzen 1980). Surprisingly it is noted by Paulo Antonio Silva, Celine Melo & Lucilene Brito that, Calyx water of *Spathodia campanulata* was used by blue and yellow macaws in Brazil (Paulo Antonio *et al. 2015)* for consumption. We know that Plants have been used as one of the important source for treating various diseases of human beings since ancient times and numbers of plants are mentioned in different traditional system of medicine but the African tulip tree, *Spathodea campanulata*, native of West African tropical forests, has been use as source of consumable water by birds is quite interesting and important. Portugal-Araujo (1963) found more than 200 dead insects (meliponine bees, flies and ants) in flowers of one inflorescence of *S. campanulata* in West Africa (PORTUGAL-ARAUJO, V., 1963). *Spathodia campanulata* is traditionally used in the treatment of various disorders. Different parts of *Spathodea campanulata* such as flowers, leaves, stem, bark and roots have been reported for possessing anti-inflammatory, analgesic, cytotoxic, anti-diabetic and anticonvulsant activity. Phytochemical screening shows the presence of various secondary metabolites like alkaloids, tannins, flavonoids, glycosides and sterols(Wagh*et al*2018). According to Jayanthi *et al* development and flower opening in *S. campanulata* is also due the influence of phytohormones present in the calyx fluid besides the endogenous concentrations of tissues as an exogenous source of hormones. In *Spathodea campanulata* floral part development occurs within water calyces, under watery fluid which is unique among flowering plants. Hence, pharmacognostical and physiochemical standardization of *Spathodia campanulata* P.Beauv. calyx water important and it was carried out.

*S. campanulata* is widely and commonly known as the African tulip tree, and has been introduced pan-tropically for its ornamental value. *Spathodea campanulata* is indigenous to Africa with a native range that extends along the west coast from Guinea to Angola, and inland across the tropical rainforest region to southern Sudan and Uganda; however, the exact limits are uncertain, and nativity in neighboring countries is possible, or it could have been introduced in the extremes of this range (Kairo M*et al*. 2003). *S. campanulata* has been successfully planted as an ornamental throughout the humid tropics, and its actual distribution is likely to be more widespread and introduction to South Asia occurred at the end of the 1800s and other introductions may have been as old.

## Material and Methods

### Material

*S. campanulata* is a tropical tree suited to humid conditions and grows well in areas with an even distribution of rainfall, though it will tolerate a dry season of up to six months. It grows on a wide variety of sites, from poorly to excessively drained, but prefers fertile, deep and well-drained loams.*S. campanulata* is a medium-size to large tree up to 35 m tall and 175 cm in diameter. Leaves are usually opposite (rarely 3 at a node), very widely diverging, up to 50 cm long, (7-) 11-15 (-17) leaflets broadly elliptic or ovate, entire, to 15 x 7.5 cm, with 7-8 principal veins on each side, puberulent and prominent beneath, petiole up to 6 cm long. Raceme are 8-10 cm long on a peduncle of about the same length, with a pair of reduced leaves about halfway up, rachis and pedicels thick, brownish puberulent, bracts subtending pedicels lanceolate, curved, about 1 cm long. calyx strongly curved upward, asymmetric, about 5 cm long, tapering, somewhat ribbed, splitting at anthesis to within a few mm of base along dorsal curve, apex horn-like, blunt, exterior brownish sericeous puberulent; corolla bright vermilion or scarlet, 10-12 cm long, orange-yellow; filaments about 5 cm long. Fruit capsules are lanceolate, slightly compressed, 17-25 x 3.5-7 cm. *S. campanulata* flowers and fruits during the dry season in some regions, for 5-6 months a year though, or in others it will flower all year round. It may begin flowering when 3-4 years old in favourable sites. It is an obligate outcrosser (Bittencourt et al., 2003).Freshly flower buds of *S. campanulata* of different flowering seasons from different trees were collected from different locations in Nasik city.

### Methods

The healthy buds were selected and grouped into small, medium and large sizes based on the size cum weight of intact flower buds. For extracting calyx fluid, unopened floral buds were washed in distilled water to remove surface contaminants. Under *in vitro* condition, the floral buds were washed in 0.1% mercuric chloride for 10 minutes followed by three washes in autoclaved distilled water for 5 minutes. The flower buds were again surface sterilized with absolute alcohol. The calyx fluid was extracted from the respective flower buds with the help of sterile syringes. For physicochemical study, calyx fluid from all the bud sizes were collected freshly, mixed and analyzed qualitatively and quantitatively for pharmacognostical and physiochemical standardization. Flowing method was used for estimation(Martin Paul*et al*, 2019)

Benedict's reagent method: To 1 ml of sample, 1 ml of Benedict's reagent wasadded and heated in a boiling water bath for 10 min. The formation of red/yellow precipitate was observed as positive reaction.

Ninhydrin method: To 1 ml of sample, 1 ml of 0.1% freshly prepared ninhydrin solution was added. The contents were mixed and boiled for 2 min, allowed to cool. The appearance of violet/purple complex was observed as positive reaction.

Estimation of reducing sugars: Reducing sugars were estimated by Nelson-Somogyi method. To 1 ml of fresh fluid, 1 ml of fresh alkaline copper tartarate reagent was added. The mixture was heated for 20 min in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was mixed and diluted to 25 ml with distilled water. The resulting blue colour was read at 620 nm using Genesys visible spectrophotometer. 1 ml of distilled water with all other reagent except sample was used as blank. Reducing sugars in the samples were calculated from glucose standards.

Estimation of total free amino acids: Total free amino acids were estimated by Ninhydrin method. To 1 ml of fresh sample, 1 ml of Ninhydrin reagent was added. The tube was heated in a boiling water bath for 20 min, cooled to room temperature. 5 ml of diluents solution (equal volume of water and n-propanol) was added and mixed. The volume was raised to 25 ml with distilled water and the intensity of purple colour was read using Genesys visible spectrophotometer at 570 nm. 1 ml of distilled water with all other reagent except sample was used as blank. Total amino acids in the fluid was calculated from glycine standards.

Estimation of proteins Proteins were estimated by Lowry's method: To 1 ml of sample 5 ml of alkaline copper solution was added mixed well and allowed to stand for 10 min at room temperature. 0.5 ml of folin-ciocalteau reagent was added, mixed and incubated at room temperature in dark for 30 min. The volume was raised to 25 ml with distilled water. The intensity of the blue coloured complex was read using Genesys visible spectrophotometer at 660 nm. 1 ml of distilled water with all other reagent except sample was used as blank. Proteins in the fluid was calculated from bovine serum albumin (BSA) standards

Estimation of total phenols: Total phenols were estimated by Folin-Ciocalteu reagent. To 1 ml of Folin-Ciocalteu reagent 1 ml of fresh sample was added, followed by 2 ml of 20% sodium carbonate. The mixture was heated in boiling water bath for one min. The volume was raised to 25 ml with distilled water and the intensity of blue color was read using Genesys visible

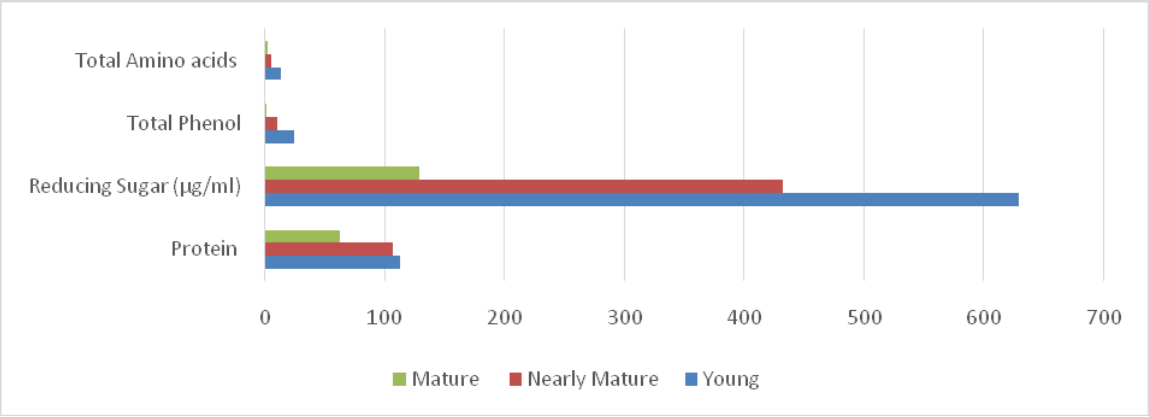
Antimicrobial tests was performed with well diffusion method, DPPH assay method was used for antioxidant test, fertilizer test was taken by general sowing method, FTIR by FTIR spectroscopy.

**Result and Discussion**

**Table showing Nutritional Analysis Results for of *S. campanulata* calyx fluid**

| Bud Stage | Bud Size(cm) | pH | EC (µS/cm) | Protein (µg/ml) | Reducing Sugar (µg/ml) | Total Phenol (µg/ml) | Total Amino acids (µg/ml) |
|---|---|---|---|---|---|---|---|
| Young | 2.5 | 8.35 | 11.65 | 112.31 | 628.28 | 24.23 | 13.12 |
| Nearly Mature | 3.5 | 8.28 | 9.46 | 106.48 | 432.32 | 10.26 | 5.3 |
| Mature | 4.5 | 8.22 | 9.09 | 62.41 | 128.52 | 0.12 | 2.12 |

**Graph Showing concentration (µg/ml) of Protein, Reducing sugar, Total Phenol, Total Amino acids at different stages of bud growth**



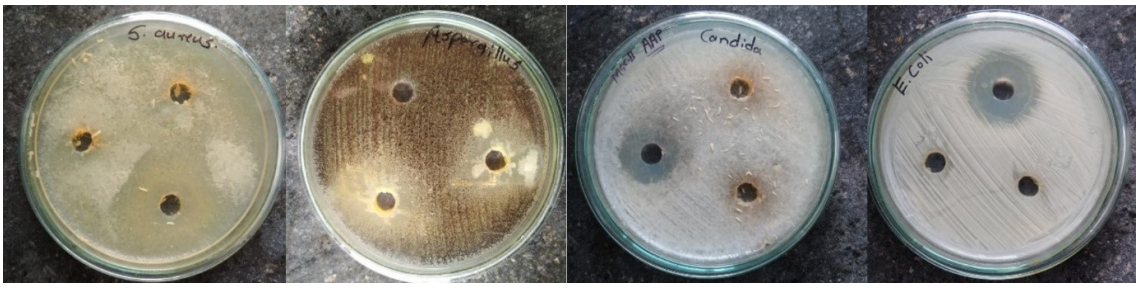

Electrical conductivity(EC), pH, concentrations of protein, reducing sugar, total phenol and total amino acid shows reducing trend as bud proceeds to maturation stage. Calyx water pH of *S. campanulata* young bud decreases gradually as bud size increases and reaches to maturity. It is noted that when bud was young pH was 8.35 and it was 8.22 at mature stage.

Same observations was seen for Electrical conductivity (EC), it was 11.65 µS/cm at young, 9.46 µS/cm at nearly mature and 9.09 µS/cm at mature stage. Concentration of protein, reducing sugar, total phenol and total amino acid at young stage was 112 µg/ml, 628.28 µg/ml, 24.23 µg/ml and 13.12 µg/ml respectively. Remarkable decrease in the all the concentration was seen as bud grows and reaches to maturity.

**Table showing antimicrobial activity of of *S. campanulata* calyx fluid**

| Test | Escherichia coli | Staphylococcos aureus | C. albicans | Aspergillus niger |
|---|---|---|---|---|
| Calys Fluid | 12 | 15 | 21 | 25 |
| Std. Glutamycin | 22 | 25 | - | - |
| Std. Fluconazol | - | - | 24 | 26 |

**Photographs showing antimicrobial activity of of*S. campanulata* calyx fluid**



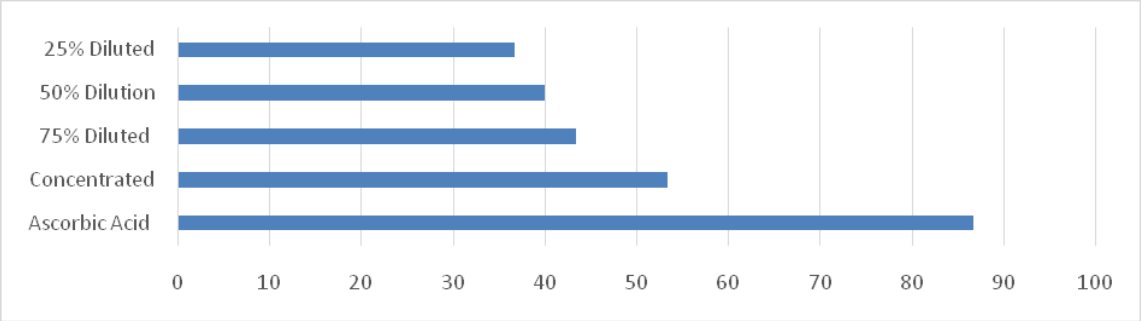

S. aureus A. niger C. albicans E.coli

We have tested antimicrobial activity of *S. campanulata* calyx fluid against E.coli, S. aureus, C. albicans and A. nigar with Glutamycin and Fluconazol as a standard antimicrobial agents. But result was quite surprising that, *S. campanulata* calyx fluid shows antimicrobial activity against all the members, while Glutamycin was effective aginst only E.coli, and S. aureus and Fluconazol was effective against only C. albicans and A. nigar. *S. campanulata* calyx fluid was almost comparable with std. Fluconazol against C. albicans and A. nigar.

**Table Showing Antioxidant activity of *S. campanulata* calyx fluid**

| Sample | Ascorbic Acid | Concentrated Calyx Fluids | 75% Diluted Calyx Fluids | 50% Dilution Calyx Fluids | 25% Diluted Calyx Fluids |
|---|---|---|---|---|---|
| Antioxidant activity of Calyx fluids (%) | 86.66 | 53.33 | 43.33 | 40.00 | 36.66 |

**Graph of antioxidant activity of *S. campanulata* calyx fluid against standard in percentage.**




*S. campanulata* calyx fluid and its different dilutions was tested for antioxidant activity using Ascorbic acid a comparative. gradual decrease in the antioxidant activity was noted as we increase the dilution of calyx fluid

**Table showing potentiation activity of *S. campanulata* calyx fluid**

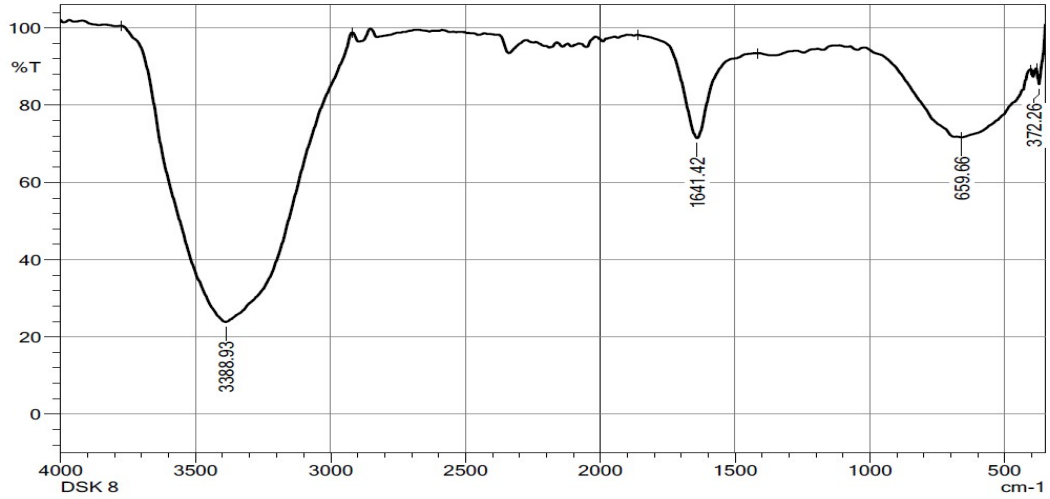
| Content | E.coli(mm) | B. subtilis(mm) | C. albicans(mm) | A. niger(mm) |
|---|---|---|---|---|
| Calyx fluid | 19 | 18 | 24 | 26 |
| Cow Urine | 19 | 18 | 20 | 24 |
| Gentamycin | 23 | 21 | - | - |
| Cow urine + Calyx fluid | 24 | 24 | 21 | 28 |
| Gentamine + Calyx fluid | 24 | 22 | - | - |
| Heated Calyx fluid | 19 | 19 | 21 | 24 |

*S. campanulata* calyx fluid tested for the potentiation effect on anti microbial agent like cow urine and Gentamycin by agar well method using the microbes like E.coli, S. aureus, C. albicans and A. nigar. The cow + and calyx fluid and Gentamine + calyx fluid shows better result than its individual activity on E.coli and S. aureus. But cow + and calyx fluid and Gentamine + calyx fluid shows no activity against C. albicans and A. nigar. Fresh and heated calyx fluid alone has good activity against C. albicans and A. nigar. These results indicate that the calyx fluid of *S. campanulata* has good potentiation effect with cow urine and Gentamycin.

**Phytohormone Test**

| Phytohormone | IAA | IBA | Siderophore |
|---|---|---|---|
| Concentration | ++ | + | ++ |

**FTIR of *S. campanulata* calyx fluid**




| No. | Peak | Intensity | Corr. Intensity | Base (H) | Base (L) | Area | Corr. Area | Comment |
|---|---|---|---|---|---|---|---|---|
| 1 | 372.26 | 85.39 | 7.90 | 383.83 | 349.12 | 332.301 | 165.268 | |
| 2 | 659.66 | 71.67 | 0.13 | 661.58 | 406.98 | 5838.490 | 849.605 | |
| 3 | 1641.42 | 71.43 | 24.42 | 1861.31 | 1417.68 | 4674.516 | 2823.303 | |
| 4 | 3388.93 | 23.82 | 75.93 | 3776.62 | 2918.30 | 36694.517 | 36408.174 | |

**Funcional group present in *S. campanulata* calyx fluid**

| Peak | Area | Functional group |
|---|---|---|
| 372.26 | 332.301 | Unknown |
| 659.66 | 5838.49 | -C=C- |
| 1641.42 | 4674.516 | -C=C- |
| 3388.93 | 36694.51 | -OH |

The spectrum revealed the existence of various functional groups of different phytoconstituents contained in the calyx fluid of ***S. campanulata***. The vibrational frequencies have been assigned to different functional groups. Calyx fluid of ***S. campanulata*** exhibited a characteristic band at 1641.42 and 3388.93 indicating the presence of carbonyl (-C=C-) and Hydroxyl(-OH) group respectively.

**Conclusion**

Calyx fluid of S. *campanulata* shows high in the concentration of reducing sugar, phenol, protein and amino acids which indicate its absolute necessity for the floral development. The concentrations of all the analyzed biomolecules were found to be decreasing with bud maturity. The contents of water calyx fluid are obligatory for the development of floral whorls. It can be said that, there is good potential in high nutritive calyx fluid can be used a

nutritional supplement and novel food source. Presence of phytohormones like IAA, IBA and Siderophore in the calyx fluid in bud maturity suggests that the calyx fluid has a vital role in the exponential development of corolla. Presence of antimicrobial activity indicates the role of calyx fluid in immunological defense mechanism of *S. campanulata*. Antimicrobial activity can be exploited in the preparation of pharmaceutical and cosmetic products like soaps and medicines. Antioxidant activity indicates a high metabolic rate and healthier growth of *S. campanulata* flower. Antioxidant activity can be exploited for the preparation of anti-ageing and creams. Calys water can be used as a potentiation agent with antimicrobial drugs as well as with cosmetics and sap water is glycoprotein in nature. Calyx water has a stimulus interaction with Gentamycin and streptomycin(Potentiation activity)

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