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Phytochemical evaluation of *Holostemma ada-kodien* Schult. A rare medicinal plant

Archana PJ
Post Graduate and Research Department of Botany & Biotechnology, Sree Narayana College, Kollam, Kerala, India

Abstract
Holostemma ada-kodien is well known for its medicinal and rejuvenative potential. A preliminary phytochemical screening and estimation of total phenols in the leaf tissue was also carried out in the present study. The present finding of this study support the view that *H. ada-kodien* is a promising source of potential antioxidants which can be used in treatment of various diseases. The qualitative analysis of alkaloid, tannin, saponin, phlobatannin, flavonoid, terpanoid, glycoside, steroid, quinone, coumarin and phenol are done. And also the quantitative estimation of total amount phenols was done by using catechol as standard.

Keywords: medicinal plant, phytochemical, qualitative

1. Introduction
Holostemma ada-kodien Schult is a handsome laticiferous twiner, provided with conspicuous flowers (Warrier, 2004) [13], and tuberous roots. According to Moming (1987) [10], Warrier *et al.* (1995) [12] and Gupta (1997) [2], the plant is used as antidiabetic, rejuvenative, aphrodisiac, expectorant, stimulant, galactogogue, ophthalmic disorders and for maintaining youthful vigour, strength and vitality. The tuberous roots of *H. ada*-kodien are medicinally important and it have huge demand in South Indian pharmacies. Roots are used as ingredient for the preparation of the drug "jivanthi" (Kolammal, 1979) [7]. The root tubers produced terpanoid sugars that are responsible for the medicinal properties (Ramiah *et al.*, 1981) [11]. *Holostemma ada-kodien* is listed as vulnerable and rare in Foundation for Revitalization of Local Health Traditions red list of medicinal plants (FRLHT, 1997) [1]. The phytochemical evaluation is essential for identification of the chemical components are present in this plant.

2. Materials and Methods
2.1 Sample Preparation
The leaves of *H.ada-kodien* were collected from field grown plants. The aqueous and ethanol extract of the leaves were prepared by homogenizing 10 g plant material in 50 ml of distilled water or ethanol respectively. The phytochemical composition of the leaf aqueous extract (LAQE) and leaf ethanol extract (LEtOHE) were screened by qualitative methods using standard protocols (Kokate, 1994, Harbone, 1973 and Marinova *et al.*, 2005) [9, 6, 3].

3. Result and Discussion
The enormous information gathered from the phytochemical evaluation of *H.ada-kodien.* This study leads to the qualitative analysis of chemical compounds found in the leaf of this plant. Preliminary phytochemical screening of the leaf aqueous extract of the plant showed positive test for alkaloids, tannins, phlobatannins, flavonoids. However saponines, coumarins and quinine could not be detected in the leaf aqueous extract .Terpanoids, steroids and glycosides were detected in the organic extract of the leaf (Table 1).

Table 1: Phytochemical screening of *Holostemma ada-kodien* Schult. leaf aqueous extract (LAQE) and leaf ethanol extract (LEtOHE).

Secondary metabolites	Presence in LAQE	Presence in LEtOHE
Alkaloids	++++	
Tannins	+++	
Saponins	-	
Phlobatanines	++	
Flavanoids	+++	
Terpanoids		++++
Glycosides		++
Steroids		Nil
Quinones		Nil
Coumarines		Nil
Phenols		++++

H.ada-kodien showed curative properties due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. Preliminary phytochemical screening of the leaf aqueous extract of the plant showed positive test for alkaloids, tannins, phlobatannins, flavonoids. terpenoids, glycosides and phenol were detected in the organic extract of the leaf. The presence of flavonoids and tannins may be responsible for the antidiabetic activity of the plant as reported by Janapati *et al*. (2008) Phenolic compounds are effective hydrogen donors, they are good antioxidants. Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Marinova *et al.* 2005) [9]. The present study also revealed the presence of phenols and flavonoids in the leaves. The therapeutic properties may be attributed to the phenolic compounds present. Irimpan *et al*. (2011) [4] carried out preliminary phytochemical screening of stem and leaf sample of the herb. The antimicrobial properties of leaf extracts such as aqueous, hydro alcoholic, ethanolic and

methanolic were tested on both gram negative and gram positive bacteria. The plants showed antimicrobial properties that compared with standard antibiotic Gentamicin.

- **Test for Alkaloid:** To 3 ml leaf aqueous extract (LAQE) was stirred 3 ml of 1% HCl, on water bath. Mayer Wagner's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

- **Test for Tannins:** To about 2 ml of the LAQE was stirred 2 ml of distilled water and few drops of $FeCl_3$ solution added. Formation of a green precipitate indicated the presence of tannins.

- **Test for Saponins:** About 5 ml of the LAQE was shaken vigorously with 5 ml distilled water in a test tube and warmed. There was no formation of stable foam, indicating the absence of saponins.

- **Test for phlobatannins:** To about 2 ml of LAQE was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

- **Test for Flavanoids:** *Lead acetate Test*: To 1 ml of LAQE, 1 ml of 10% lead acetate solution was added. The formation of a yellow coloured precipitate was taken as a positive test for flavonoids.

- **Test for Terpenoids:** *Chloroform Test*: Two ml of the leaf ethanol extract (LEtOHE) extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

- **Tests for glycosides:** *Liebermann's test*: 2 ml of the LEtOHE was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of glycoside.

- **Tests for steroids:** A red colour was not produced in the lower chloroform layer when 2 ml of LEtOHE was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the absence of steroids.

- **Tests for Quinones:** To 1 ml of extract 1 ml of concentrated sulphuric acid was added absence of red colour shows the absence of quinones.

- **Test for Coumarins:** No yellow colour formation on adding 3 ml sodiumhy droxide (10%) to 2 ml of extract indicates the absence of coumains.

- **Test for Phenols:** To 1 ml of the extract, 2ml of distilled water was added followed by 1-4 drops of 1% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

- **Phenol estimation:** Estimation of total phenols was done by the method of Malick and Singh (1980). Leaf samples were homogenized in 80% ethanol and centrifuged at 10,000 rpm. Supernatant collected in china dishes and evaporated to dryness in a hot air oven at 60 ºC. The dried supernatant extracted in a known volume of distilled water. Aliquots were taken and made up to 1 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) and 20% sodium carbonate were added. The tubes placed in hot water bath for 1 min cooled and absorbance read at 650 nm. Catechol was used as standard and the total phenols expressed as mg g^{-1} f. wt. of tissue.
  The phenolic content of the leaves was estimated to be 14.01 mg g^{-1} fresh weight of tissue.

4. Conclusion

The result of the present study suggests that *H.ada-kodien* has various secondary metabolites and has good quantity of total phenols. But at this point it is not known what components in the plant extracts are responsible for the known activities of this plant. Detailed studies on chemical composition, isolation of active constituents and pharmacological evaluation are essential to characterize them as biological antioxidants. The present finding of this study support the view that *H.ada-kodien* is a promising source of potential antioxidants which can be used in treatment of various ailments.

5. References

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