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Systematic validation of anti-inflammatory activity of raw drug samples in *Holostemma annulare* (Roxb.) K. Schum

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The tuberous roots of *Holostemma annulare* are utilized as the drug *Jivanti* in Ayurvedic medicine system. There is a huge demand of root tubers of this plant by pharmacies. Conversely, there are reports concerning adulteration in market samples of *Jivanti* resulting in damaging effect on the quality of drug formulations. Till now there is no significant study to relate the source plants available in markets as *Jivanti*. A meticulous phytochemical profiling especially of the roots is still a lacuna and no studies have been carried out yet regarding this. We focused on this concept and analyzed the anti-inflammatory activity by means of proteinase inhibition assay, as well as COX and LOX inhibition assays in the root samples collected from homestead cultivation (*HS*) and from an authenticated trade shop in Thiruvananthapuram (*TS*). Among the two samples, *TS* exhibited comparable anti-inflammatory activity to *HS* which further confirms the authenticity of the genuine drug in the preparation of Ayurvedic formulations. The study provides a scientific rationale in using *Holostemma* roots in traditional drug preparations for diseases linked with inflammation and also throw light in fortifying molecular approaches in validating elite raw drugs in order to supplement genuine samples for pharma needs.

Keywords: Anti-inflammatory, COX, *Holostemma annulare,* LOX, Proteinase, Tuberous roots

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The word 'inflammation' has origin from the Latin world *Inflammare* which means a state of being inflame or heat associated with redness and swelling and it is a physiological response that protects the body from tissue injury[1]. Inflammatory condition is correlated with various pathological conditions *viz.*, atherosclerosis, sepsis, cancer, arthritis and metabolic syndromes[2] and it may be either acute or chronic inflammation. Acute inflammation occurs few minutes after tissue damage, while the failure of managing acute inflammation and a self-directed response to a self-antigen lead to chronic inflammation and diseases. The acute inflammation is mediated by serotonin, histamine and COX-2 and the chronic inflammation by PGE2, Nitric oxide and Lipoxygenase products. Cycloygenase (COX) is the key enzyme in the synthesis of prostraglandins, prostracyclins and thromboxanes which are involved in inflammation, pain and platelet aggregation[3]. Steroidal and non-steroidal drugs like Aspirin, Ibuprofen, Diclofenec, Ketoprofin and Naproxen are current medicaments in dealing acute inflammatory diseases. The continuing usage of these drugs result in antagonistic side effects and mutilate human biological systems such as liver, gastrointestinal tract, etc. and causes renal failure in addition to gastro intestinal damage. This demands the efforts to formulate alternative anti-inflammatory drugs from natural ingredients with least adverse effects. Quite a lot of medicinal plants have considerably contributed to the drug discovery and still providing ample source for lead molecules as drug candidates. Till to date, more than hundreds of publications are there regarding the anti-inflammatory activities of plants[4].

The species targeted for the study *Holostemma annulare* (Roxb.) K. Schum. (Syn. *Holostemma ada-kodien* Schult (Family Asclepiadaceae), a medicinal plant chiefly distributed in the Western Ghats is used in the customary system of medicine for maintaining youthful vigour and potentiality. The root tubers of this plant are medicinally important and are worthwhile in ophthalmopathy, orichitis, cough, fever, burning sensation, stomachalgia and also as expectorant, tonic, stimulant and galactagogue[5]. Roots of *Holostemma* are

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used as ingredient for the preparation of the drug 'jivanti'[6] in the indigenous system of medicine and are used for diabetes[7]. According to Khare[8], Charaka and Bhavaprakasha described *Jivanti* as Shakashreshtha (the best among leafy vegetables), an agent for spermatogenesis and galactagogue by Sushruta, and according to Dhanvantari Nighantu and Kaiyadeva Nighantu it is known to promote vision. The leaves, flowers and fruits are eaten as vegetable and the root is used in many ayurvedic formulations such as 'Jeevanthyadi ghritham', 'Aswaghandhadi ghritham', 'Jeevanthyadi yamakam', 'Balaristam', 'Anuthailam', 'Punarnavabaladi kashayam', 'Chandanadi thailam', 'etc.[9]. Traditionally the plant is used as an alternative, astringent to the bowels; cures ulcers, diseases of the blood, worms[10], itching, leucoderma; useful in gonorrhoea as tonic and stomachic, aphrodisiac agent[11].

The therapeutic properties of *Holostemma* spp. were contributed by the terpenoid sugars existing in the root tubers of the plant[12]. There is a huge demand of more than 150 tonnes of root tubers of this taxa every year by the South Indian pharmacies. The indiscriminate and ruthless collection of the root tubers in recent times has led to acute scarcity of the plant and is recently listed out as an endangered species in Kerala[13]. All over Kerala and mostly in South India, *Holostemma ada-kodien* Schult is used as source plants of *Jivanti* and there are reports about adulteration in market samples, which will cause an undesirable impact in the quality of medicines prepared. There is also a growing practice of consuming substitute herbal raw drugs, in cases where the authentic herbal raw drug is not available in required quantities. Till now there is no significant study to compare the source plants available in markets as *Jivanti*. We here describe a detailed phytochemical evaluation as well as screening of some bioactive potentialities especially the anti-inflammatory activity of the raw drug *Jivanti* collected from homestead cultivation (*HS*) and the samples from authenticated raw drug markets of Thiruvananthapuram (*TS*). It also unraveled the possible mechanism in the treatment of inflammatory disorders and substantiated the proven therapeutic use of this plant in traditional medicine for inflammatory diseases.

Materials and Methods

Collection of plant material

Holostemma annulare root samples (from two years old plants) were collected during 2018 from homestead cultivation at Vembayam, Thiruvananthapuram, Kerala, India as well as from an authorized raw drug shop (Santhigiri Ayurveda Pharmacy) in Thiruvananthapuram, Kerala, India (from about five years old plants) (*HS* and *TS* respectively).

Preparation of plant extracts

The collected roots were washed well, dried and powdered, 25 g of powdered plant material was subjected to soxhlet extraction for eight hours using methanol as the solvent. The extract was concentrated to dryness and the residue (~30 mg) was transferred to a sample bottle and was stored for further studies.

Phytochemical screening

Phytochemical tests for alkaloids (Dragendroff's test and Wagner's test), flavanoids (Ammonium test, Alkaline reagent test, Shinoda test), phytosterols/ terpenoids (Liebermann-Burchard's test), tannin and phenol (Ferric chloride test), triterpenoids (Salkowski test), anthraquinone glycosides, carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test), saponin (Foam test) were carried out as according to the standardized procedures by Harborne[14] and Sofowora[15].

Quantitative analysis of phyto-constituents *viz*. carbohydrates by Anthrone method[16], reducing sugar by DNS method[17], proteins[18], tannins[19], alkaloids[15], phenols[20], flavanoids[21] and triterpenoids[22] were carried out in the methanolic extract of root samples (homestead cultivation samples and trade samples) of *H. annulare*.

In vitro anti-inflammatory activity assays

In vitro anti-inflammatory activity of the two selected samples was evaluated by analyzing the proteinase inhibitory activity, COX and LOX activities.

Proteinase Inhibitory Activity

Proteinase inhibitory activity of the methanolic root extracts was performed according to the method of Sakat *et al*[23]. Different concentrations of sample such as 62.5-500 µg mL^{-1} from a stock concentration of 10 µg mL^{-1} were used for the study. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL 20 Mm Tris HCl buffer (pH 7.4) and 1 mL test sample at different concentrations. The mixture was incubated at 37ºC for 5 min. Then 1 ml of 0.8% (w/v) casein was added and incubated for an additional 20 min. Then 2 mL of 70% perchloric acid was added to

terminate the reaction. Cloudy suspension was centrifuged at 3000 rpm for 10 min. The absorbance was measured using uv-visible spectrophotometer at 200 nm (SL119, Systronics) against the buffer as blank. The percentage of proteinase inhibitory activity was calculated using the following formula,

$$\% \text{ inhibition} = 100 - \frac{[OD_{\text{Test solution}} - OD_{\text{Product control}}] \times 100}{OD_{\text{Test control}}}$$

Cycloxygenase* (*COX*) *and Lipoxygenase* (*LOX*) *assays

Cell lines

RAW 264.7 cells were grown to 60% confluence followed by activation with 1 µL lipopolysaccharide (LPS) (1 µg mL^{-1}). LPS stimulated RAW cells were exposed with different concentration of sample solution. Diclofenac sodium, a standard anti-inflammatory drug in varying concentration corresponding to the sample was also added. After 24 h incubation, the anti-inflammatory assays were performed using the cell lysate.

Cycloxygenase (*COX*) *assay*

The COX activity was assayed by the modified method of Walker and Gierse[24]. The cell lysate in Tris-HCl buffer (pH 8) was incubated with glutathione (5 mM l^{-1}) and hemoglobin (20 µgL^{-1}) for 1 min at 25°C. The reaction was initiated by the addition of arachidonic acid (200 mM l^{-1}) and terminated after 20 min of incubation at 37°C, by the addition of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 1% thiobarbiturate, COX activity was determined by reading absorbance at 632 nm. Percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100$$

Lipoxygenase (*LOX*) *assay*

The determination of 5-LOX activity was carried out as per Axelrod *et al*.[25]. Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL; 10 mg mL^{-1}). The LOX activity was monitored as difference in absorbance at 234 nm, which reflects the formation of 5-hydroxyeicosatetraenoic acid from linoleate. Percentage inhibition of the enzyme was calculated as mentioned above.

Statistical analysis

All the experiments were conducted in six replicates and was repeated thrice. Data were presented as mean±SE and were statistically analysed based on one way ANOVA and the mean values were compared by student t-test performed in Microsoft Excel at $p \leq 0.01$.

Results

Phytochemical analysis

Phytochemical screening revealed the presence of various phyto-constituents in both samples of *H. annulare*. Methanolic root extracts of homestead samples (*HS*) indicated the presence of most of the phytochemicals *viz*., alkaloids, flavanoids, triterpenes, tannins, phenols, proteins, phytosterols with exception of saponins and anthraquinones (Table 1). Observations indicated that the alkaloids, phenols and flavanoids were significantly higher in the homestead samples (*HS*) compared to the drug from shop (*TS*).

Quantitative analysis of phyto-constituents *viz*. carbohydrates, reducing sugar, proteins, tannins, alkaloids, phenols, flavanoids and triterpenoids carried out in the methanolic extract of root samples revealed that the *HS* roots of *H. annulare* contained relatively high amount of soluble sugar (12.68 mg g^{-1}) compared to the protein and reducing sugar contents (0.986 mg g^{-1} and 0.080 mg g^{-1} respectively). The results indicated that alkaloid content in root extracts from homestead cultivation (*HS*) and trade samples (*TS*) were 7.62 and 6.43 mgg^{1} respectively. The total flavanoid content was also moderately high in homestead cultivation sample (1.88 mg g^{-1}) than in the trade sample (1.51 mg g^{-1}). Similarly slightly high phenolic content was noticed in home stead cultivation extract (10.47 mg g^{-1}) than in trade sample (10.38 mg g^{-1}). The concentration of tannin estimated as tannic acid equivalent (TE mg g^{-1}) was recorded to be 0.88 mg g^{-1} in the trade sample and 0.42 mg g^{-1} in homestead sample (Table 2).

In comparison to alkaloids, flavanoids, phenolics and tannin, the total triterpenoid content detected was substantially low in methanolic extract of trade sample (0.285 mg g^{-1}) than the homestead sample and was found to be in the order *HS* triterpenoid (0.312 mg g^{-1}) >*TS* triterpenoid (0.285 mg g^{-1}) (Table 2). The results indicated that the root sample collected from homestead cultivation was comparatively better source of phyto-components than that of trade sample; whereas the tannin content was higher in *TS* than *HS*. The amount of phyto-constituents varied in little

Table 1 — Qualitative analysis of phytochemicals in roots samples of *H. annulare*

Test for phytochemicals	Observation	Methanol (*HS*)	Methanol (*TS*)
Alkaloids:			
Wagner's test: Two drops of Wagner's reagent was added to 1 mL of the test solution along the side of the test tube.	Formation of yellow or brown precipitate	+++	++
Dragendroff'stest: 1 mL of Dragendroff's reagent was added to 2 mL of the filtrate along the side of the test tube.	Formation of a reddish brown precipitate	++	++
Flavanoids:			
Alkaline test: A small quantity of extract was heated with 10 mL of ethyl acetate in boiling water bath for 3 min. The mixture was filtered differently and the filtrate was shaken with 1 mL of 1% (v/v) dilute ammonia solution. The layers were allowed to separate.	A yellow colour observed at ammonia layer	+	++
Shinoda test: A few magnesium turnings and 5 drops of concentrated hydrochloric acid was added drop wise to 1 ml of test solution.	A pink, scarlet, crimson red or occasionally green to blue colour appeared after few minutes	+++	+
Triterpenoids:			
Salkowski test: Approximately 2 mg of dry extracts was shaken with 1 mL of chloroform and a few drops of concentrated sulfuric acid was added along the side of test tube.	A red brown color formed at the interface	++	+
Tannin:			
$FeCl_3$: A few drops of 5% (w/v) ferric chloride solution was added to 2 mL of the test solution.	Formation of bluish black colour	+	+
Phenol:	Formation of greenish black colour	+	+
Extracts were treated with 3-4 drops of 10% (w/v) ferric chloride solution			
Antraquinones:	Appearance of Red colour	-	-
To 1 mL of the extract, a few drops of 10% (w/v) potassium hydroxide solution was added			
Carbohydrates:			
Molisch's test: To 1 mL of the test solution a few drops of 1% α-naphthol and 2- 3 mL concentrated sulphuric acid was added along the side of test tube	A reddish violet or purple ring at the junction of two liquids	++	+
Fehling's test: 2 mg dry extract was dissolved in 1 mL of distilled water and 1 mL of Fehling's (A and B) solution was added, shaken and heated on a water bath for 10 min.	Brick red precipitate	-	-
Saponins:			
Foam test: 5 mL of the test solution was taken in a test tube and shaken well for five minutes.	Stable foam	-	-
Proteins:			
Biurette test: To 2 mL of the test solution 5 drops of 1% (w/v) copper sulphate solution and 2 mL of 10% NaOH was added, and mixed thoroughly.	Formation of purple or violet color	+	+
Phytosterol:			
Libermann-burchardstest: 2 mg of the extract was dissolved in 2 mL of acetic acid anhydride, heated to boiling, cooled and then 1 mL of concentrated sulphuric acid was added along the test tube side.	A brown ring formation at the junction	++	-

(+++ High; ++ Moderate; + Low; - Nil)

extent in both the samples and the variation was statistically significant only at 1% level of significance and the minute variation can be correlated to some other factors *viz.* the maturity of the samples, the time of harvesting the raw drug, or the extraction procedures and so on.

***In vitro* anti-inflammatory activity assays**

Proteinase inhibitory activity

Proteinase inhibitory activity of *H. annulare* root samples is shown in Fig. 1. The standard used was Diclofenac sodium and the OD of test control was 0.9113. The inhibition levels were observed within

Table 2 — Quantitative analysis of phytochemicals in *H. annulare* root samples

Phytoconstituents	Quantity (mgg^{-1})	
	HS	*TS*
Carbohydrate	12.68±0.01^{a}	11.81±0.08^{b}
Reducing sugar	0.80±0.01^{a}	0.78±0.08^{ab}
Proteins	0.986±0.002^{a}	0.929±0.001^{b}
Alkaloids	7.62±0.06^{a}	6.43±0.15^{b}
Phenols	10.47±0.05^{a}	10.38±0.08^{a}
Flavanoids	1.88±0.05^{a}	1.51±0.03^{b}
Tannin	0.42±0.01^{b}	0.88±0.02^{a}
Triterpenoid	0.312±0.009^{a}	0.285±0.001^{b}

*Data represents mean values of six replicates repeated thrice. Mean values followed by the same letter in the superscript in a row do not differ significantly based on ANOVA and t- test at $p \leq 0.01$.

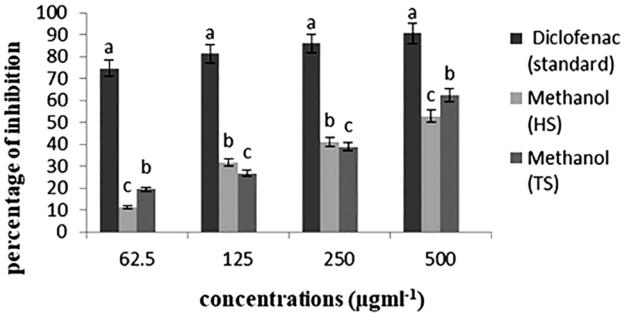

Fig. 1 — Proteinase inhibitory activity of *H. annulare* samples

the range of 11.2-63.13%. The *TS* samples showed significantly higher proteinase inhibition as follows: *TS* 62.52%, IC_{50} = 368.836 µg mL^{-1} and *HCS* 52.84%, IC_{50} = 425.576 µg mL^{-1}. Here, significantly high inhibition was noticed at the lowest and highest concentration tested in *TS* samples than *HS*.

Cycloxygenase* (*COX*) *assay

The anti-inflammatory effects of *H. annulare* root extracts on the production of prostaglandin'swere estimated by determining the percentage inhibition of cycloxygenase activity. The *HS* and *TS* disclosed strong inhibitory activity of the COX enzyme with IC_{50} values 89.19 and 79.65 mg mL^{-1}, respectively. At various concentrations *viz.* 6.25, 12.5, 25, 50, 100 µg mL^{-1}, the percentage of inhibition for COX enzyme exhibited by standard Diclofenac sodium were 10.32, 26.97, 36.45, 52.85 and 60.75% with an IC_{50} value of 52.98 mg mL^{-1}. The percentageof COX inhibition exhibited by different concentrations (6.25, 12.5, 25, 50, 100 µg mL^{-1}) of *HCS* and *TS* were found to be 12.26, 14.71, 20.75, 26.60 and 59.24% (IC_{50} = 89.19 mg mL^{-1}) then 8.6, 22.16, 30.4, 46.7 and 54.94% (IC_{50}= 72.27 mg mL^{-1}) correspondingly (Fig. 2). In this

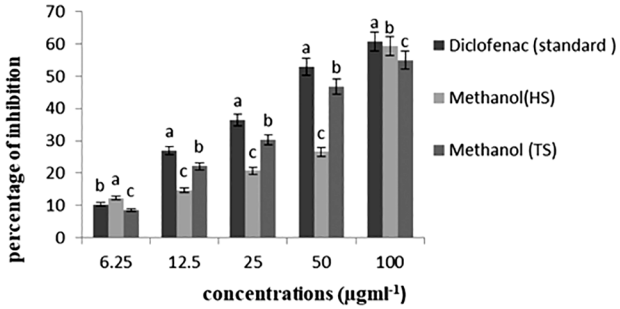

Fig. 2 — Cycloxygenase inhibition activity of *H. annulare* samples

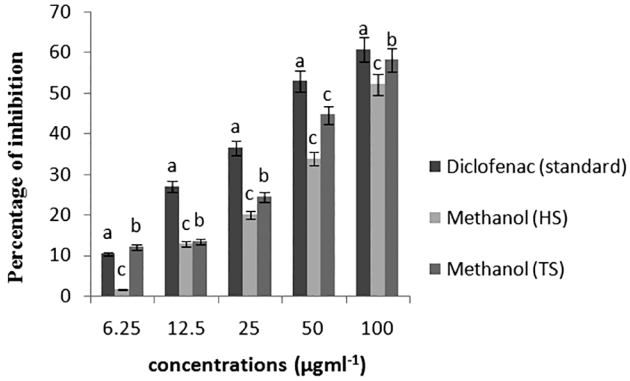

Fig. 3 — Lipoxygenase inhibition (LOX) Assay of *H. annulare* samples

assay comparatively less activity was recorded for the lowest and highest concentration (6.25 and 100 µg mL^{-1}) while the moderate concentration exhibited better inhibition (Fig. 2).

Lipoxygenase* (*LOX*) *assay

The anti-inflammatory effects of *H. annulare* root extracts such as *HCS* and *TS* on the production of leukotrienes were estimated by inhibition of lipoxgenase activity. In our study *TS* extract showed better 5-lipxoygenase inhibitory activity when compared to the control and standard. The results indicated that each extracts of the *H. annulare* roots were found to be effective against inflammation in a dose dependent manner. The activity of methanolic root extracts of *TS* was found more effective as compared to *HCS*.

In LOX assay, the percentage of inhibition at various concentrations of root extracts of *TS* (6.25, 12.5, 25, 50, 100 µg mL^{-1}) was found to be 11.97, 13.45, 24.42, 44.61 and 58.07%, while for the standard Diclofenac sodium, the percentage of inhibition at the same concentrations were 10.32, 26.97, 36.45, 52.85 and 60.75%, respectively (Fig. 3). The highest inhibitory effect was observed for *TS* with an IC_{50} value 71.07 mg mL^{-1}. Among the two

extracts, *TS* has higher LOX inhibiting activity than *HCS*. The percentage of inhibitory activity *HCS* extract against COX enzyme (IC_{50}= 89.19 mg mL^{-1}) were generally higher to that of LOX (IC_{50}=84.91 mg mL^{-1}) at the same concentration. The inhibitory effect of root extracts on the enzymesfollow a dose dependent action. Screening of the two sample extracts at 100 mg mL^{-1} concentration demonstrated this particular concentration had the best potential to inhibit both COX and LOX.

Discussion

Inflammation is a well-coordinated biological process, brought in by microbial infection or tissue injury[26] and can be controlled efficiently by modulating inflammatory mediators such as kinins, cytokines, adhesion molecules like ICAM and VCAM prostaglandins and leukotrienes. Inflammatory diseases include different types of rheumatic disorders such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and osteoarthritis[27]. In acute inflammation, the activated cellular phospholipases causes the breakdown of membrane phospholipids into arachidonic acid, which in turn is metabolized by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX) to secondary inflammatory mediators, such as prostaglandins and leukotrienes respectively[28]. Thus, COX and LOX plays important roles in the regulation of inflammatory response. Cycloxygenases (COX) or prostaglandin endoperoxide H synthases are enzymes that catalyze a bisoxygenase reaction leading to the production of important biological mediators called prostanoids which includes prostaglandins, prostacyclins and thromboxanes[29]. It has two catalytic activities, a cyclooxygenase activity in which arachidonic acid gets converted to prostaglandin G2 (PGG2) and a peroxidase activity in which PGG2 undergoes a two electron reduction to PGH2 which is then processed to various classes of bioactive lipids like thromboxanes, PGF2a, PGD2, PGI2 and PGE2[30]. The over expression of cyclooxygenase may leads to serious problem especially inflammation, malignant tumors of the colon and rectum[30]. So its inhibition is indispensible for the treatment of inflammation and cancer. Lipoxygenases (LOXs) catalyzes the dioxygenation of polyunsaturated fatty acids in lipids containing a cis-1,4-pentadiene structure to produce leukotrienes, that are lipid signaling molecules derived from arachidonic acid (AA) which initiate and amplify innate and adaptive immune responses by regulating the recruitment and activation of leukocytes in inflamed tissues[31]. Likewise cyclooxygenase, LOX enzyme metabolites also produce severe problems related to inflammation, augment metastasis of tumor cells and stimulate tumor cell adhesion[32,33]. The search for inhibitors of these enzymes thus forms the basis for exploring the medicinal taxa for the development of new anti-inflammatory agents from it.

In the present study, phytochemical screening of the two samples of *H. annulare* showed the presence of terpenoids, alkaloids, flavonoids, etc. which might have contributed significantly towards the biological activities such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities, etc. The bioactive phytochemicals like phenolics, flavonoids, terpenoids, etc., act as antioxidants which prevent oxidative cell damage caused by free radicals[34]. The high anti-inflammatory activity in terms of high percentage of inhibition in COX and LOX activity of the plant samples recorded here may be due to the presence of these phytochemicals. Among these phytocomponents, the flavonoids have proven anti-inflammatory mechanisms by means of their ability to impede generation of reactive oxygen or nitrogen compounds. They have also been recommended to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells, or in brain cells after a stroke[29]. Flavonoids have many pharmacological activities and have contributed significantly to the antioxidant and anti-inflammatory activity of *Holostemma* plants[35,36]. The *HS* and *TS* samples of the *H. annulare* showed relatively higher amount of flavonoid content (1.88 mg g^{-1}, 1.51 mg g^{-1} respectively). The total triterpenoid content noticed was 0.285 mg g^{-1} in *TS* and 0.312 mg g^{-1} in *HS*. The established analgesic and anti-inflammatory activity of terpenoids[37] hereby substantiates the medicinal use of *H. annulare* roots in terms of its anti-inflammatory potential.

Thus the anti-inflammatory activity of root extract of *H. annulare* has been attributed to the inhibition of the synthesis of prostaglandins (PGs) and leukotrienes

due to the presence of flavonoids and terpenoids which act as free radical scavengers and in that way inhibiting inflammation; besides the detailed mechanism of action needs to be ruled out. The findings confer that phyto-constituents in *H. annulare* root extract were capable of inhibiting COX and LOX enzyme of the arachidonic acid cascade in human cellular system. Supporting this, the ethanolic extract of leaves of *H. ada-kodien* holds good anti-inflammatory activity against albino rats[38]. Moreover, the ethanolic extract of latex protease of *H. ada-kodien* also has superior anti-inflammatory activity[39]. Among the two samples analyzed, *TS* exhibited comparable anti-inflammatory activity than *HS* which further ratifies the authenticity of the genuine drug in the preparation of ayurvedic formulations. The *HS* also revealed anti-inflammatory effects but the slight superiority of the *TS* samples may be attributed to the variation that arise due to some other factors *viz*. the maturity of the samples, the time of harvesting the raw drug, eco-climatic factors or the extraction procedures and so on which needs further molecular marker approaches for selection of elite materials. In Ayurveda, the traditional system of Indian medicine, herbal formulations or combined plant extracts are used in the treatment of a wide variety of ailments and clinical application of this approach is found to be successful as conventional treatment[40]. The study also throw light on this aspect of medicament in utilizing root tuber extracts of *Holostemma* for treating disorders linked with inflammation.

Conclusion

The anti-inflammatory activity of the two root samples of *Holostemma* were evaluated by examining the proteinase inhibitory activity along with the COX and LOX assays. The determined activity increased in the samples with the increase in volume of the extracts and the outcomes provides a scientific rationale for the use of *Holostemma* roots in traditional drug formulations in the treatment of diseases linked with inflammation. The present investigation also confirm that the raw drug collected from the drug shop for the assays exhibits slightly superior activity than the home stead samples and this might be due to morphogenetic diversity of this species with respect to different geographical distribution. Hence, a molecular approach is inevitable to reinforce the phytochemical validation of the samples collected from different eco-geographical zone and it would be an advantage to introduce unique clone having qualitative and quantitative improved traits for cultivation and consistent utilization according to commercial demand in phyto-pharma industry.

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Conflict of Interest

Author declare that they have no conflict of interest.

Authors' Contributions

TSP has designed the experiment, wrote the final manuscript and provided overall supervision of the work, AP and SPS conducted the experiments and wrote the manuscript draft. All authors read and approved the final manuscript.

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