IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

PHYTOCHEMICAL ANALYSIS OF DIFFERENT SOLVENT EXTRACTS OF *COSTUS IGNEUS*.

Ratnakar Subhash More[1] , Prakash Sudhakar Baviskar[2]

[1 2]B.P.Arts S.M.A. Science and K.K.C. Commerce College, Chalisgaon
Email- rsmore97@gmail.com,

Abstract :

Phytochemicals are present in medicinal plants. *Costus igneus* is used as a medicinally important plant for its anti-diabetic property. The purpose of the current study was to choose the best extraction solvent. The powder of roots, stem and leaves were separately extracted with different solvents like ethanol, methanol, chloroform, acetone and distilled water. Tannins, phlobatannins, alkaloids, phenols, saponins, flavonoids, terpenoids, triterpenes, steroids, cardiac glycosides, proteins, carbohydrates, reducing sugars were present in roots, leaves and stem of *Costus igneus*. Ethanolic extract shows most of the phytochemicals are present in *Costus igneus*.

Key words : *Costus igneus*, phytochemicals, alkaloid, diabetes,

Introduction:

Phytochemicals occur naturally in medicinal plants and protect them from various diseases. Phytochemical studies have gained a lot of interest among the scientists due to the development of new technology. (Muthukumar, C.M., Cathrine, L., & Gurupriya, S., 2019). A plant synthesizes a different chemical substance that induces great interest due to their versatile applications. It is estimated that 14-18% of the higher plants are used for medicinal purposes and out of those 74% of pharmacologically active plants were discovered after following up on ethno-medicinal usage of the plants.

Costus igneus is used in India to control diabetes and it is known that diabetic people eat one leaf daily to keep their blood glucose low (Devi V.D., Urooj A.,2008). The plant belongs to the family Costaceae. The Costaceae was first raised to the rank of family by Nakai on the basis of spirally arranged leaves and rhizomes being free from aromatic essential oils (Hegde, P. K. et al, 2014). *Costus igneus* is also known as fairy costus or spiral flag or insulin plant. It contains a range of phytochemicals viz flavonoids, Tannin, Phlobatannin, Saponin, Steroid, Terpenoids, Cardiac glycosides. In this study qualitative, quantitative phytochemical analysis were conducted.

Materials And Methods

Collection of sample

Plant materials were collected from personal garden of my guide Dr. P. S. Baviskar.

Preparation of Sample:

Leaf, stem and root of *Costus igneus* were cleaned and shade-dried. The each dried part of *Costus igneus* were crushed by a mechanical grinder separately and passed through a mesh.

Extract preparation:

Powdered samples were separately extracted with different solvents like acetone, ethanol, methanol, hexane and distilled water using soxhlet apparatus and were used for further analysis.

Phytochemical Analysis:

1) **Test for tannins**

   Ferric Chloride Test: 2.0 ml of extracts were taken then 2.0 ml of $FeCl_3$ was added. Intense blue or brownish green colour precipitate indicates the presence of tannin in plant extracts.

2) **Test for alkaloids**

   Wagner's Test: 2.0 ml of extracts were taken, add 2.0 ml of Wagner's reagent (2.5 gm of Iodine is dissolved in 12.5gm of potassium iodide) solution in it. If intense brownish colour forms, this indicates the presence of alkaloids in plant extract.

3) **Test for Saponins**

   Foam Test: 2.0 ml of plant extracts were taken, add 20 ml distilled water. The test tube was then shaken for 15 minutes. The formation of layer of foam indicats the presence of saponins in plant extracts.

4) **Test for Cardiac Glycoside**

   Keller-Killani Test: 5 ml of extract is treated with 2 ml of glacial acetic acid, containing one drop of $FeCl_3$ solution and 1 ml of conc. H_2SO_4. Browning of the interface indicates a deoxysugar

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

which is the characteristic of cardiac glycosides. Below the brown a violet ring was observed while in the acetic acid layer a greenish ring was observed.

5) **Test for Phenol**

Ferric Chloride Test: In the extract add few drops of $FeCl_3$ solution. Appearance of intense brown colour indicates the presence of phenol in plant extract.

6) **Test for Flavonoid**

Alkaline Reagent Test: 2.0 ml of extracts were taken then add 0.5 ml of NaOH solution in it. If intense yellow colour that becomes colourless on addition of few drop of diluted HCl indicated the presence of flavonoid in the plant extract.

7) **Test for steroids**

In 2.0 ml of aqueous plant sample were taken then and 2 ml of chloroform solution was added in it and add 2ml of conc. H_2SO_4 from the side of the test tube. Formation of intense yellow colour with green fluorescence colour indicates the precipitate of steroid.

8) **Test for phlobatannins**

2.0 ml of plant extract add 2 ml of 1% HCl solution and it was then kept for boiling water bath. Presence of phlobatannine is indicated by red colour precipitate.

9) **Test for terpenoid**

Salkowski test: In 2.0 mL of extracts add 2 ml of chloroform and 0.5 ml of conc. H_2SO_4. Formation of intense reddish-brown colour indicates the precipitate of terpenoid.

10) **Test for Triterpenes**

For qualitative analysis of triterpenes, 2.0 ml of extracts were taken. To these extracts, 1 ml of conc. H_2SO_4 solution was added. Formation of intense brown ring indicates the precipitate of triterpene.

11) **Test for Protein**

In 2.0 ml of plant extracts add 2.0 ml of Ninhydrin reagent and boiled for 5 to 10 minutes in boiling water bath**.** Formation of dark purple colour indicates the presence of protein in plant extract.

12) **Test for carbohydrate**

Molisch Test : In 2.0 ml of plant extract add 0.5 ml of H_2SO_4 solution and these extracts, 2 ml of Molisch reagent was added. Formation of intense violet ring indicates the precipitate of carbohydrate.

13) **Test for Reducing sugar**

Fehling's Test: In 2.0 ml of plant extracts add 2 ml each of Fehling A and Fehling B solution were added and boiled for 5 minutes. Formation of orange red colour indicates the presence of reducing sugar.

Quantitative Analysis of *Costus igneus* :

➢ **Determination of Tannin**

- **Standard graph preparation of Tannic acid:**

Prepare aqueous solution of tannic acid of the concentration of 0.1 mg/ml. Remove six aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml and transfer it into 100ml volumetric flask. Add 5ml of Folin-Ciocalteu phenol reagent and add 10ml of aqueous solution of sodium carbonate and makeup the volume of 100 ml incubate it for 30 min at room temperature and measure the absorbance of each sample in spectrophotometer at 760 nm.

- **Extract preparation :**

Add 25ml of 80% methanol in 500 mg of dried sample and heat it for 30 min. filter it and wash the filtrate with 80% methanol to avoid loss. Make the volume 50ml through volumetric flask.

- **Total Tannin determination**

Take 3 ml of sample and add 5 ml of Folin-Ciocalteu phenol reagent and add 10ml of aqueous solution of sodium carbonate and makeup the volume of 100ml incubate it for 30 min at room temperature and measure the absorbance of each sample in spectrophotometer at 760nm. Plot the graph of standard tannic acid concentration versus absorbance. Plot absorbance of sample on standard graph and calculate the concentration of tannin present in extract.

➢ **Flavonoid determination**

- **Preparation of Standard**

Prepare the methanolic solution of rutin as a standard with a concentration of 0.5 mg/ml. Remove six aliquots of 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 ml, and create final concentrations of 6.0, 8.0, 10.0, 12.0, 16.0 and 20.0 µg/ml of rutin, respectively. Add 0.6 ml of glacial acetic acid, 10 ml of the pyridine solution and

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

2.5 ml of the aluminum chloride reagent. Makeup the volume with distilled water, incubate for 30 min at room temperature. measure the absorbance of each sample in spectrophotometer at 420 nm.

- **Extract preparation :**

Add 25ml of 80% methanol in 500 mg of dried sample and heat it for 30 min. filter it and wash the filtrate with 80% methanol to avoid loss. Make the volume 50ml through volumetric flask. Repeat all steps in triplicate.

- **Quantification procedure**

Transfer 1 ml of the plant extract in to the 25 ml volumetric flask add 0.6 ml of glacial acetic acid, 10 ml of pyridine solution and 2.5 ml of aluminium chloride reagent make up the volume with distilled water and incubate at room temp for 30 min. Complete the volume with purified water and wait 30 min take readings in the spectrophotometer at 420 nm.

➢ **Phenol Determination :**

The stock solution of phenol (10^{-1} mol/l) was prepared by dissolving a certain amount of solid phenol in distilled water. The working solutions were prepared by dilution from the stock solution with distilled water. The ferric chloride solution was obtained by dissolving 1.61 g of solid $FeCl_3$ in 100 ml of distilled water. One drop of HCl solution (36%) was added to the ferric chloride solution, before diluting it to the appropriate volume, to prevent the secondary hydrolysis. Spectrophotometric measurements were performed with Spectrophotometer at 540nm.

Phenol determination is based on the reaction between phenol and ferric chloride in aqueous media, when a soluble purple reaction product is obtained,

➢ **Saponin Determination :**

The samples were grinded then 20mg of each was put into a conical flask and 100ml of aqueous ethanol was added. The samples were heated at about 55°C in a water bath for 4 hrs with continuous stirring. The mixture was filtered, and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40 ml in the water bath at about 90°C. The concentrate was transferred into a 250ml separator funnel then 20ml of ethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. The purification process was repeated again. 60ml of n-butanol were added to the aqueous layer. The combined n-butanol extracts were washed twice with 10ml of aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in an oven to a constant weight. The saponin content was calculated as a percentage (Obadoni and Ochuko, 2001).

RESULTS

Qualitative analysis of phytochemicals in leaf, stem and rhizome of *Costus igneus*:

The presence of various compounds viz., tannins, phlobatannins, alkaloids, phenols, saponins, flavonoids, terpenoids, triterpenes, steroids, cardiac glycosides, proteins, carbohydrates and reducing sugars were analysed in ethanol, methanol, chloroform, acetone and distilled water extracts of the leaf, stem, and root of *Costus igneus*. All the mentioned compounds were present in the ethanol extract of leaves than the others extracts (Table-1).

Quantitative analysis of phytochemicals in leaf, stem and rhizome of *Costus igneus*:

The leaves of *Costus igneus* contains higher amount of alkaloid, phenol, flavonoids, steroids, while compare to leaf and stem as shown in the Tables 1.

DISCUSSION

The present study was conducted with the objective to identifying the best extraction solvent that can be used to extract the maximum amount of phytochemicals from the dried *Costus igneus* plant (stem, leaves and root). Qualitative biochemical estimations were conducted to detect the presence of different phytochemicals in the dried *Costus igneus* plant leaves extracts obtained by using different solvents i.e., methanol, acetone, chloroform, ethanol and distilled water. Our results highlight that all the extracts formed by using different solvents from the *Costus igneus* plant leaves, stem and root contain phytochemicals like tannins, phlobatannins, alkaloid, phenol, saponins, flavonoids, terpenoids, triterpenes, steroids, cardiac glycosides, proteins, carbohydrates and reducing sugars.

The fresh leaves, roots and stem of *Costus igneus* were extracted with different solvent such as methanol, acetone, chloroform, ethanol and distilled water. Among the different extracts, ethanol extract contained most of the compounds such as alkaloids, phenols, flavonoids, steroids, carbohydrates and reducing sugar. Quantitative phytochemical analysis revealed that alkaloid, phenol flavonoid and steroids were high in leaves than stem and root. The present study focused on the qualitative and quantitative characterization of phytochemical components of *Costus igneus*

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

CONCLUSION :

A comparative study has been conducted with the aim of achieving the best extraction solvent for the extraction of phytochemicals from *Costus igneus* plant leaves. The results from this study demonstrate that using ethanol as an extraction solvent results in the maximum extraction of phytochemicals in leaves (tannins, phlobatannins, alkaloids, phenols, saponins, flavonoids, terpenoids, triterpenes, steroids, cardiac glycosides, proteins, carbohydrates and reducing sugars). Chloroform and distilled water results in the least extraction of different phytochemicals, which may be due to the poor solubility of these phytochemicals and should not be the solvent of choice.

To the best of our knowledge this is the first report that directly compares five extraction solvents and our results clearly demonstrates that ethanol is the best extraction solvent for the extraction of various phytochemicals from the leaves of *Costus igneus* plant. This can be explored further.

REFERENCES :

1. Jose, Beena, and L. J. Reddy. **"Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant Costus pictus from southern India."** *International Journal of Pharmacy and Pharmaceutical Sciences* 2, pp.100-101 · May 2010.
2. Hegde, P. K., Rao, H. A., & Rao, P. N. (2014). **A review on Insulin plant (Costus igneus Nak).** *Pharmacognosy reviews*, *8*(15), 67–72. https://doi.org/10.4103/0973-7847.125536.
3. Muthukumar, C.M., Cathrine, L., & Gurupriya, S. (2019). **Qualitative and quantitative phytochemical analysis of Costus igenus leaf extract.** *Journal of Pharmacognosy and Phytochemistry*, *8*, 1595-1598.
4. Devi V.D., Urooj A. (2008). **Hypoglycemic potential of Morus indica. L and Costus igneus. Nak.--a preliminary study.** Indian J Exp Biol. 2008 Aug;46(8):614-6. PMID: 18814491.
5. Baris O, Gulluce M, Sahin F, Ozer H, Kilic HH, Ozkan H, Sokmen M, Ozbek T. **Biological activities of the essential oil and methanolic extract of *Achillea biebersteinii* Afan. (Asteraceae) *Turk** J Biol.* 2006;30:65–73.
6. Abdullah Mohammed Aladhreai, Dr. Shazia Jamal, (2019), **Analytical Study of Different Solvents for Phytochemical Extraction Potential from Costus igneus (stem, leaves, root).** International Journal of Scientific & Engineering Research Volume 10, Issue 1, January-2019, ISSN 2229-5518.
7. Krishnan, K., Mathew, L. E., Vijayalakshmi, N. R., & Helen, A. (2014). **Anti-inflammatory potential of β-amyrin, a triterpenoid isolated from *Costus igneus***. *Inflammopharmacology*, *22*(6), 373–385. https://doi.org/10.1007/s10787-014-0218-8.
8. Devi, G.M.R. (2021), **Quantitative phytochemical and chromatographic analysis of phenolic compounds in methanolic leaf extract of *Costus pictus* D. Don.** *JPC-J Planar Chromat* **34**, 437–446 (2021). https://doi.org/10.1007/s00764-021-00128-2.
9. Rao, Nirmala & Goud Gajula, Rajesh & Sujatha, E & Kumari, Oshi. (2016). **Phytochemical Analysis and Antimicrobial activity of *Costus igneus* (Insulin Plant) Leaf extract.** International Journal of Pharma and Bio Sciences. 5. 1207-1214.
10. Baris O, Gulluce M, Sahin F, Ozer H, Kilic HH, Ozkan H, Sokmen M, Ozbek T. **Biological activities of the essential oil and methanolic extract of *Achillea biebersteinii* Afan. (Asteraceae)** *Turk J Biol.* 2006;30:65–73.

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

Extracts	Plant part	Tannins	Saponins	Alkaloids	Phenol	Flavonoids	Cardiac Glycosides	Steroids	Phlobatanins	Terpenoids	Triterpenes	Protein	Carbohydrate	Reducing sugar
		1	2	3	4	5	6	7	8	9	10	11	12	13
Acetone	Leaf	-	+	+	-	+	+	+	+	+	+	-	+	+
	Stem	-	-	+	+	+	+	+	+	+	+	-	+	+
	Root	-	-	-	+	+	-	+	+	-	-	-	+	+
Ethanol	Leaf	+	+	+	+	+	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	-	+	+	+	+	+	+	+
	Root	+	-	+	+	+	+	-	-	-	-	-	+	+
Methanol	Leaf	+	-	+	+	+	+	-	+	+	+	+	+	+
	Stem	+	+	-	+	+	-	+	-	+	+	-	+	+
	Root	+	+	-	+	+	-	+	-	+	+	-	+	+
Chloroform	Leaf	-	-	+	-	-	-	+	-	-	-	-	+	+
	Stem	-	-	+	+	-	+	+	-	+	+	-	+	+
	Root	-	-	+	+	-	-	-	-	-	-	-	+	+
Distilled water	Leaf	+	+	+	+	-	+	+	+	-	-	+	+	+
	Stem	+	-	+	+	+	-	+	-	-	-	+	+	-
	Root	-	-	+	+	+	+	-	-	-	-	+	+	-