Analytical Study of Different Solvents for Phytochemical Extraction Potential from Costus igneus (stem, leaves, root).

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Abstract

The phytochemicals present in the Costus igneus plant is known to be responsible for its medicinal properties, to cure many diseases like diabetes . However, The present study was undertaken to compare the effect of using different extraction solvents to extract the active components like tannins, phlobatannins, saponin flavonoids, steroids, terpenoids and cardiac glycosides from the dried leaves ,stems and roots of the Costus igneus plant**. Methodology**: To achieve this, different extracts from the plant leaves, stems and roots were made using soxhlet apparatus. The extraction solvents used were acetone, ethanol, distilled water, hexane and methanol. Phytochemical estimations, total phenol concentration, flavonoid concentration and antioxidant activity have been evaluated to compare the efficiency of different extraction solvents. **Results**: The results shows that using ethanol as extraction solvent works best for the extraction of various active phytochemicals as such Tannins, Phlobatannins, aiis 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 of Costus igneus plant leaves which can be usefull in further.

Key Words: Costus igneus, Tannins, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Cardiac glycosides

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1.INTRODUCTION

Costus igneus, commonly known as fiery costus, Step ladder or Spiral flag or Insulin plant, is native to South and Central America . This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant. (Jose B and Reddy, LJ 2010.) It is widely grown in gardens as ornamental plant in South India and also run wild in many places. (Benny M ,2004;) It 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 VD, 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. Costus igneus also known as fiery costus or spiral flag or insulin plant belonging to the costaceae family, contains a range of phytochemicals viz flavonoids, Tannin, Phlobatannin, Saponin, Steroid, Terpenoids, Cardiac glycosides, and it was traditionally used in India to control diabetes(Devi and Urooj, 2008) In this study qualitative, quantitative phytochemical analysis were conducted.

Although, the usefulness of this plant is well known in medicine, as antimicrobial ,anti -diabetes . The present study was undertaken to study of different extraction solvents in extracting the different phytochemicals from Costus igneus dried plant leaves, stems and roots. The authors here for the first time report that ethanol is the best extraction solvent and can be used for the extraction of phytochemicals from the Costus igneus plant (leaves ,stems and roots).

2.MATERIALS AND METHODS

2.1.Materials: In this study, all the chemicals process were provided by a B.S.Abdur Rahman Crescent Institute of Science and Technology. including acetone, ethanol, distilled water, hexane and methanol.

2.2.Cultivation of Costus igneus

As it is shown in the Figure 2.2.1 of costus igneus, the plant was growing in the pot with the potting mix of 1:1:0.2 (redsoil:sand:vermicompost).Regular watering is done sufficient for the plant to grow in under 50% shade net condition.

[Figure 2. 1] . Costus igneus

2.3.Collection of the plant sample (Costus igneus)

The plant was taken from the Mumbai city .Leaf,stem and root of Costus igneus were collected, cleaned and shade-dried. The dried each part of Costus igneus were pulverized by a mechanical grinder and passed through a 20-mesh

[Figure 2.2.].

Plant powder(1 leaf, 2 stem ,and 3 root)

2.4.Extract preparation

A powdered samples(520g) were separately extracted with different solvents like acetone, ethanol, distilled water, hexane and methanol were done using soxhlet apparatus. Briefly, for every 150 mL of the each solvent, 13 g of the crushed plant leaves ,stem and root powder were used for soxhlet extraction. After extraction for 3 consecutive days, the crude liquids were placed in water bath at 55°C for excess solvent evaporation and it is used for further analysis (.(Kameshwara et al., 2003)

.[Figure 2.3].

(water, acetone, hexane , methanol ,acetone ,and ethanol)

3.Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens to check the presence of different phytochemicals such as flavonoids, saponins, steroids, Terpenoids, Cardiac glycosides, Phlobatannin and tannins in the Costus igneus plant extract by using standard procedures to identify the constituents as described by (Sofowara , 1993; and Harborne 1973).

3.1Qualitative analysis of phytochemicals in leaf, stem and root of Costus igneus:

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by (Sofowara , 1993; and Harborne 1973).

3.1.1.Test for tannins

About 0.5g of the powdered samples was boiled in 20ml of water in a test-tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or blue-black colorations.

3.1.2.Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

3.1.3.Test for saponin

About 2g of the powdered sample was boiled in 2ml of distilled water in the water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent

froth. The frothing was mixed with 3drops of olive oil and shaken vigorously, then observed in the formation of emulsions.

3.1.4.Test for flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated Sulphuric acid . A yellow coloration was observed inextracindicated the presence of flavonoids. The yellow coloration disappeared on standing.

3.1.5.Test for steroids

Two ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml sulphuric acid. The color changed from violet to blue or green in some samples indicating the presence of steroid.

3.1.6.Test for Terpenoids (Salkowski test)

5ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was found to show positive results for the presence of terpenoid.

3.1.7.Test for Cardiac Glyosides (Keller-Killani test)

Five ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. It was under layed with 1ml of conc. sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, greenish ring may form just gradually throughout thin layer.

3.2.Quantitative analysis of phytochemicals in leaf, stem and rhizome of Costus igneous

3.2.1.Tannin determination by Van-Burden and Robinson (1981) method

500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filter ate was pipetted out into a test tube and mixed with 2ml of 0.1M FeCl3 in 0.1N HCl and0.008M potassium ferrocyanide. The absorbance was measured at 120nm within10min using a spectrophotometer (UV 1800pc, Mapada).

3.2.2.Saponin Determination

The samples were ground and 20mg of each was put into a conical flask and 100 cm3 of aqueous ethanol were added. The samples were heated over a water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40 ml over the water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20ml of ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. 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 were dried in oven to constant weight. The saponincontent was calculated as percentage (Obadoni and Ochuko, 2001).

3.2.3.Flavonoid determination

10g of the plant sample was extracted repeatedly with 100ml of 80%aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No: 42 (125mm). The filtrate was later transferred into 45crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipai-Abyazan, 1974).

4.RESULTS

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

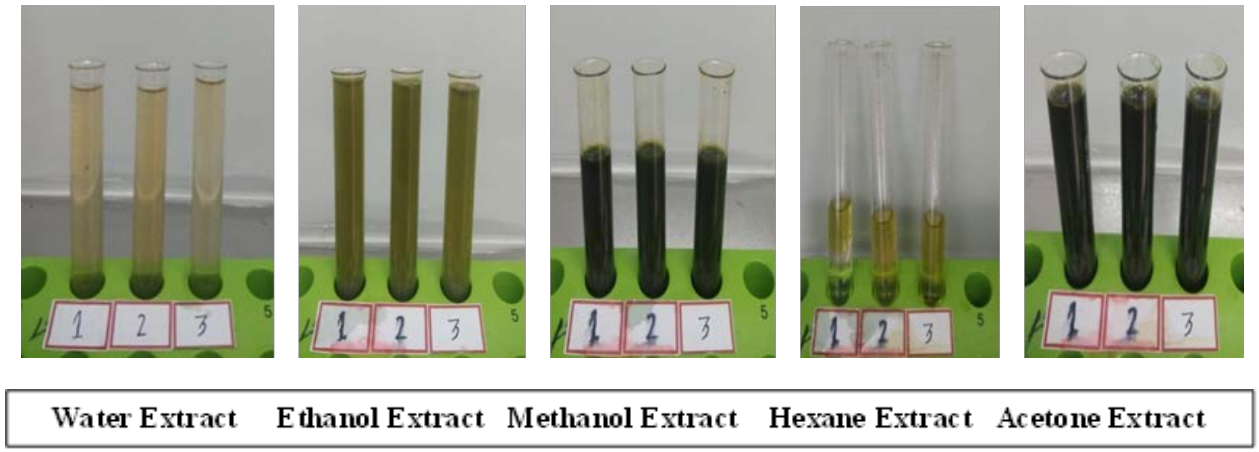
Presence of various compounds viz., tannins, phlobatannins, saponin, flavonoids, terpenoids, Steroid ,cardiac glycosides were analyzed in ethanol ,methanol, hexane, acetone ,distilled water and extracts of leaf, stem, and root and of Costus igneus. All the above mentioned compounds were present in ethanol extract than the others extracts (Table 1).

Table4.1. Qualitative phytochemical analysis of different extract of Costus igneus leaf, stem and root:

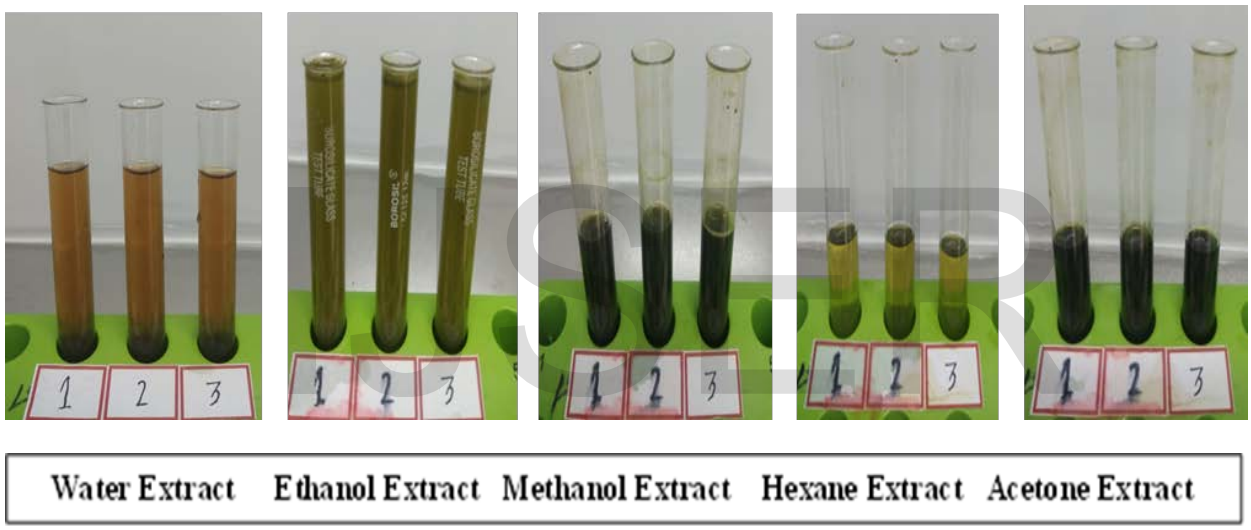
S.No	Extracts	Plant parts	1	2	3	4	5	6	7
1	Acetone	**Leaf**	+	+	+	–	+	+	–
		Stem	+	+	+	–	+	+	–
		Root	+	+	+	–	+	+	–
2	Ethanol	**Leaf**	+	+	+	+	+	+	+
		Stem	+	+	+	+	+	+	+
		Root	+	+	+	+	+	+	+
3	Methanol	**Leaf**	+	+	–	–	+	+	–
		Stem	+	+	–	–	+	+	–
		Root	+	+	–	–	+	+	–
4	Distilled Water	**Leaf**	+	+	–	+	–	–	–
		Stem	+	+	–	+	–	–	–
		Root	+	+	–	+	–	–	–
5	Hexane	**Leaf**	–	–	–	+	+	+	–
		Stem	–	–	–	+	+	+	–
		Root	–	–	–	+	+	+	–

1.Tannin, 2. Phlobatannin, 3. Saponin, 4. Flavonoid, 5. Steroid, 6. Terpenoids, 7. Cardiac glycosides.

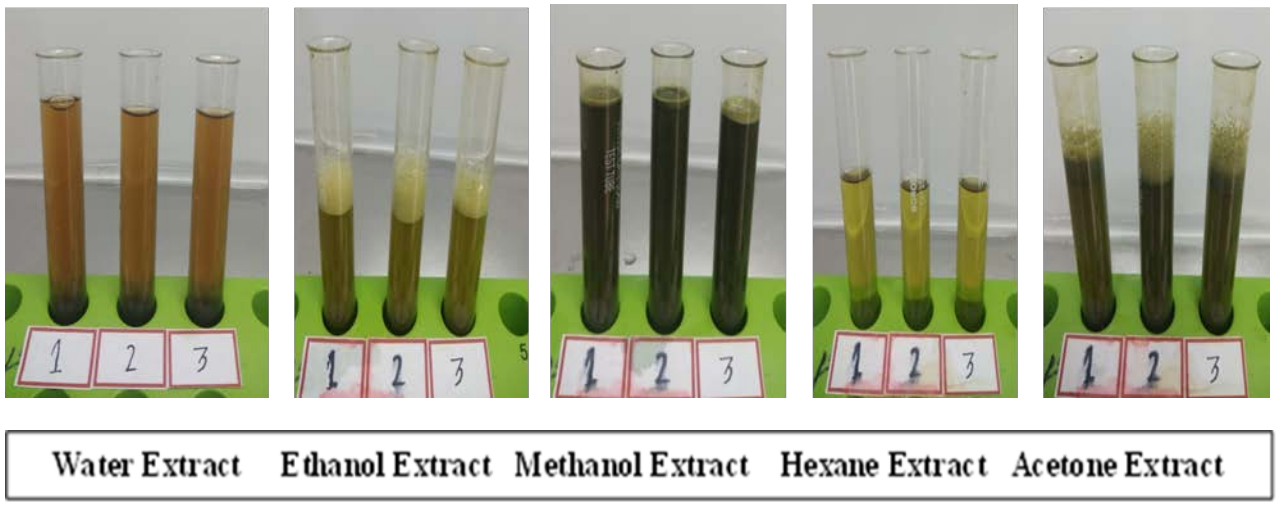
.[Fig 4.1]. Test for tannins with different extract of Costus igneus (leaf, stem and root)



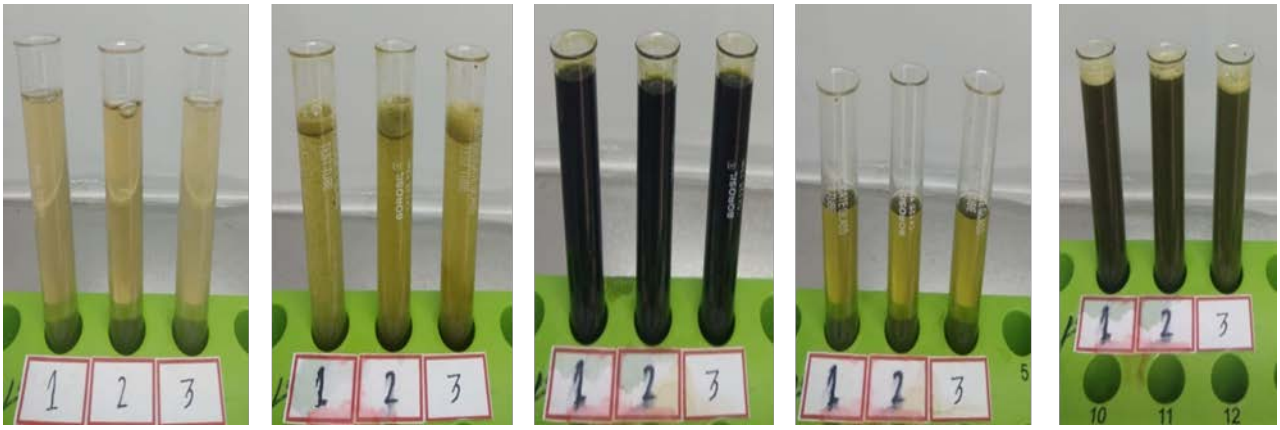
.[Fig 4.2]Test for Phlobatannin with different extract of Costus igneus(leaves, root and stem)



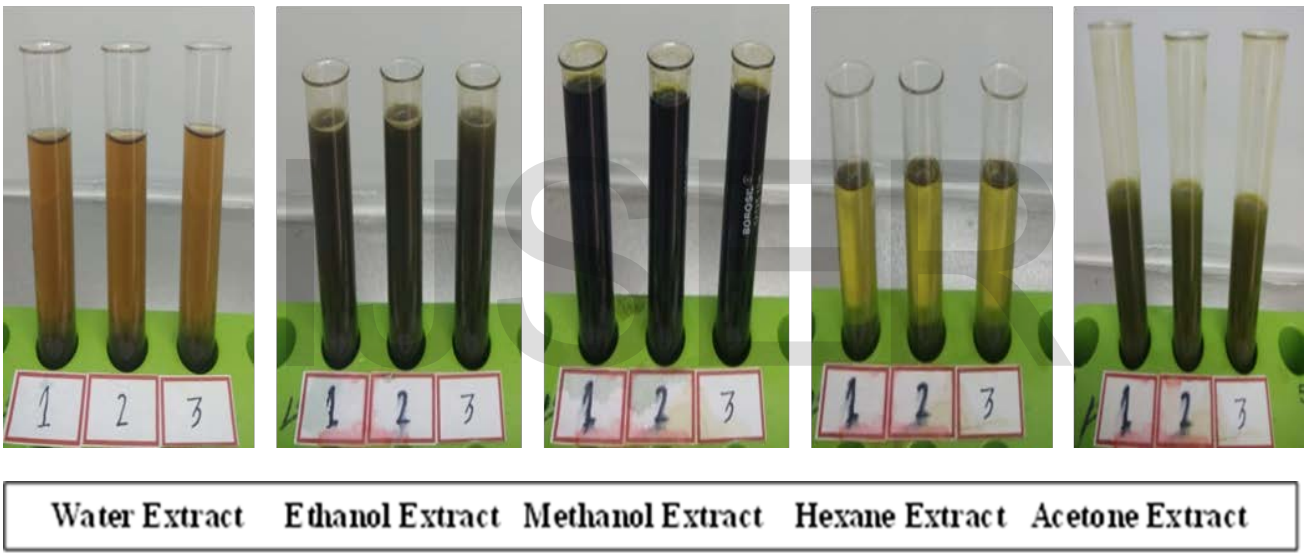
[Fig 4.3]Test for Saponin with different extract of Costus igneus(leaf, stem and root)



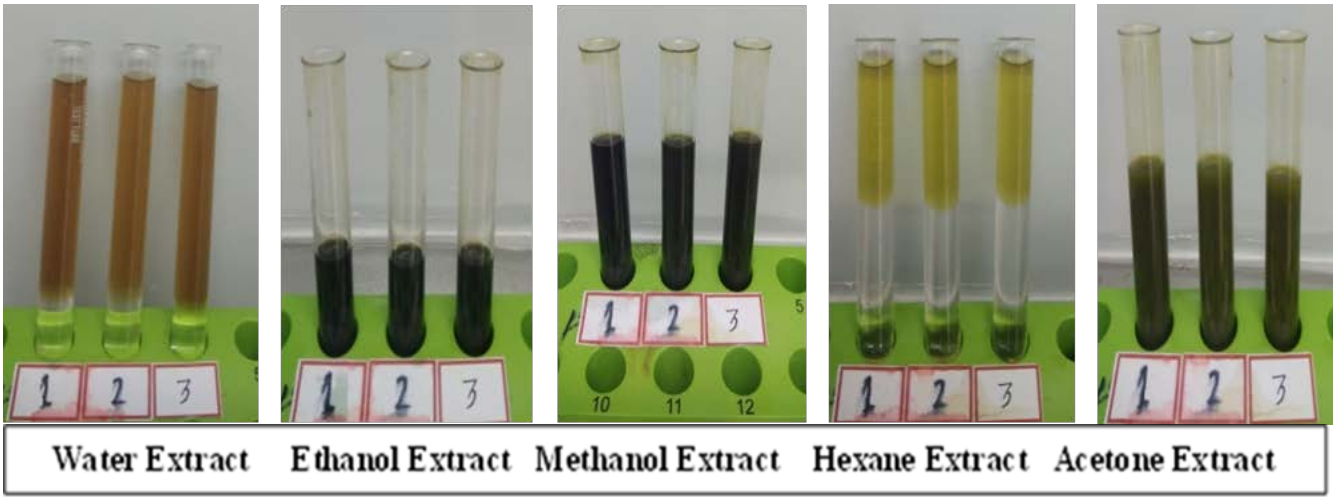
[Fig 4.4]Test for Flavonoid with different extract of Costus igneus(leaf, stem and root)



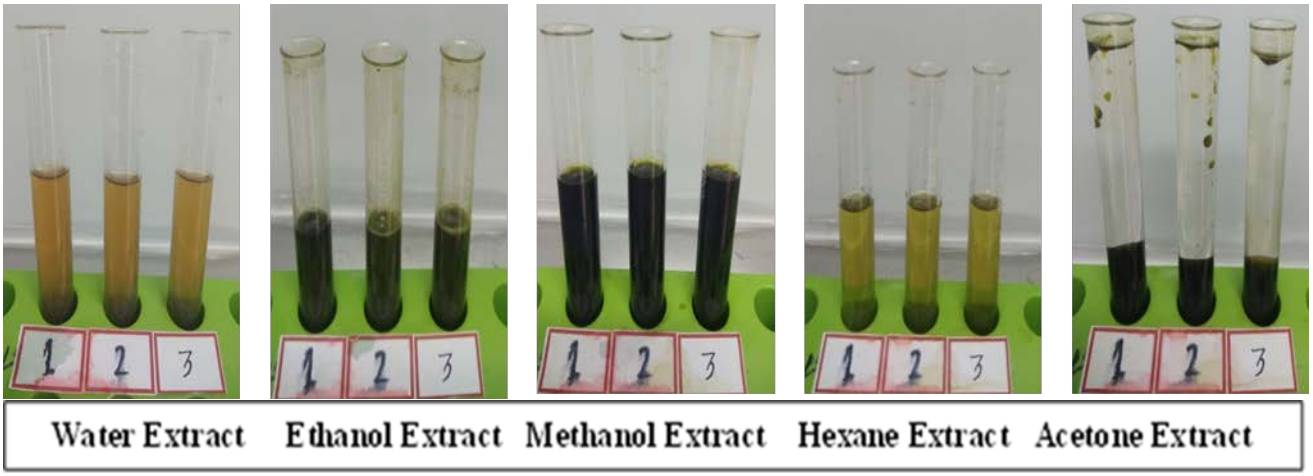
[Figure 4.5]Test for Steroid with different extract of Costus igneus(leaf, stem and root)



[Fig 4.6]Test for Terpenoids with different extract of Costus igneus(leaf, stem and root)



[Fig 4.7]Test for Cardiac glycosides with different extract of Costus igneus (leaf, stem ,root)



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

The root of Costus igneus contains higher amount of tannins, phlobatannins, saponin flavonoids, steroids, terpenoids, cardiac glycosides while compare to leaf and stem as shown in the Tables 5.2.

Table4. 2 . Quantitative phytochemical analysis of Costus igneus leaf, stem and root

S.No	Constituents	Amount (mg/kg)		
		root	Leaf	Stem
1	Tannins	**3.1**	**2.2**	**1.9**
2	Phlobatannins	**2.0**	**1.8**	**1.4**
3	Saponin	**2.4**	**2.0**	**1.3**
4	Flavonoids	**9.0**	**6.6**	**4.8**
5	Steroids	**1.7**	**1.0**	**0.98**
6	Terpenoids	**4..5**	**3..5**	**2.3**
7	Cardiac glycosides	**2.0**	**1.1**	**0.88**

5.DISCUSSION

The present study was conducted with an objective to identify the best extraction solvent, which can be used to extract the maximum amount of the phytochemicals from the dried of Costus igneus plant (stem ,leav and root). Qualitative biochemical estimations were conducted to detect the presence of different phytochemicals in the dried Costus igneus plant leave's extracts obtained by using different solvents i.e., methanol. acetone, hexane, ethanol ,and distilled water. Our results highlights that all the extracts formed by using different solvents from Costus igneus plant leaves ,stem and root contains phytochemicals like tannins, phlobatannins, saponin,flavonoids, terpenoids, Steroid ,cardiac glycosides

The fresh leaf, rood and stem of Costus igneus were extracted with different solvent such as , acetone, hexane, ethanol, methanol and water. Among the different extracts, ethanol extract contained most of the compounds such as Tannins, Phlobatannins, Saponin, Flavonoids, Terpenoids and CardiacGlycosides. Quantitative phytochemical analysis revealed that Saponin and Flavonoids were high in rhizome than leaf and stem. Present study focused on Qualitative, . Quantitative and characterization of phytochemical components of Costus igneus

A quantitative study was further conducted to detect the amount of the total flavonoids, Tannin, Phlobatannin, Saponin, Flavonoid, Steroid, Terpenoids, Cardiac glycosides and in these plant extracts for direct comparison and have demonstrated that extract ethanol contains the maximum amount of phytochemicals in leaf, stem and root .

6.CONCLUSION

A comparative study has been conducted with an aim to achieve the best extraction solvent for the extraction of phytochemicals from of Costus igneus plant leaves. The results from this study demonstrate that using ethanol as extraction solvent results in the maximum extraction of of phytochemicals in leaf, stem and root (flavonoids,Tannin, Phlobatannin, Saponin, Flavonoid, Steroid, Terpenoids, Cardiac glycosides). Hexane and distilled water results in the least extraction of various phytochemicals, may be due to poor solubility of these phytochemicals in hexane and distilled water and should not be the solvent of choice.

To 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 of Costus igneus plant leaves. This can be explored further.

7.ACKNOWLEDGMENTS

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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. Benny, M. Insulin plant in gardens. *Natural product radiance* 3(5), pp 349-350, 2004.
3. Urooj, Asna. "Hypoglycemic potential of Morus indica. L and Costus igneus. Nak.—A preliminary study." *Indian Journal of Experimental Biology*, vol. 46. pp 414-416. August 2008.
4. Kripa, K. G., D. Chamundeeswari, J. Thanka, and C. Uma Maheswara Reddy. "Effect of hydroalcoholic extract of aerial parts of Leucas aspera (Willd.) Link on inflammatory markers in complete Freund's adjuvant induced arthritic rats." *International Journal of Green Pharmacy (IJGP)* vol. 4, no. 4 October-December 2010,doi: 10.4103/0973-8258.74139.
5. Shetty, Kateel G., Jacqueline V. Huntzicker, Kathleen S. Rein, and Krish Jayachandran. "Biodegradation of polyether algal toxins–Isolation of potential marine bacteria." *Journal of Environmental Science and Health Part A,* vol.45, no. 14 pp 1850-1857 Decembe 2010, doi: 10.1080/10934529.2010.520510.
6. Bhatt, S. S., S. G. Chovatiya, and A. R. Shah. "Evaluation of raw and hydrothermically processed Prosopis juliflora seed meal as supplementary feed for the growth of Labeo rohita fingerlings." *Aquaculture nutrition*, vol.17, no. 2, pp 164-173, March 2011, doi: 10.1111/j.1365-2095.2009.00745.x.
7. Saraswathi, R., Upadhyay Lokesh, R. Venkatakrishnan, R. Meera, and P. Devi. "Isolation and biological evaluation of steroid from stem of Costus igneus." *Journal of Chemical and Pharmaceutical Research* vol. 2, no. 5, pp.444-448 2010, url : http://jocpr.com/vol2-iss5-2010/JCPR.
8. Rao, B. Kameswara, P. Renuka Sudarshan, M. D. Rajasekhar, N. Nagaraju, and Ch Appa Rao. "Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats." *Journal of Ethnopharmacology* vol.85, no. 1, pp169-172 March 2003, doi:10.1016/S0378-8741(02)00396-3.
9. Sofowora, A. *Medicinal plants and traditional medicine in Africa*. John Wiley and sons LTD, 1982.