

QUALITATIVE PHYTO-CONSTITUENTS SCREENING AND QUANTIFICATION ANALYSIS USING WITH LEAF SOURCE OF KYDIA CALYCINA ROXB. A TRADITIONAL MEDICINAL TREE TAXON.

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ABSTRACT

Background: Fossil records clearly demonstrate that humans have used plants for medicinal purposes since the middle Palaeolithic age, around 60,000 years ago (Farnsworth, 1966). A medicinal plant is defined as any plant whose one or more organs contain substances that can be effectively utilized for therapeutic purposes or serve as precursors for the synthesis of valuable drugs. These plants are abundant in bioactive components that play a pivotal role in treating various human ailments and are essential to the healing process. The medicinal properties of plant species are directly linked to their phytochemical constituents. Therefore, phytochemical screening is an essential and strategic step in identifying the bioactive compounds found in specific medicinal plants. *Kydia calycina* belongs to the family Malvaceae and well known its medicinal importance.

Results: The present study was undertaken to screen the phytochemicals with polar (Aqueous, Methanol, Ethanol and Acetone) and non-polar (Chloroform and Petroleum ether) solvents found in the leaf. Phenolic compounds found in the leaf extracted with polar solvents and not found in non-polar extracts. Ethanol is the ideal solvent to extract maximum number of metabolites. Tannins are found high levels among secondary metabolites in leaf of *Kydia calycina* the findings may be useful for identification of *K. calycina* is the best medicinal plant to use for further validation studies. **Conclusion:** The results of this study hold significant promise for pharmacologists and phyto-chemists in the identification of novel active compounds. This research may contribute to the development of innovative therapeutic agents, thereby enhancing the efficacy of various medicinal applications. This work aims to provide valuable insights into their medicinal properties, potentially leading to advancements in pharmaceutical treatments and solutions.

KEYWORDS

INTRODUCTION

Medicinal plants have played a vital role in maintaining human health and enhancing the quality of life for thousands of years. The use of plants as medicines dates back to the dawn of human civilization, with countless species of higher plants documented for their therapeutic properties worldwide. Currently, approximately 70% of the global population relies on herbal medicines, predominantly in developing countries, where these remedies are favoured for primary health care due to their cultural acceptance and perceived compatibility with the human body. The World Health Organization (WHO) estimates that 80% of the world's inhabitants rely on traditional medicine for their primary health care needs, with the majority of these therapies involving the use of plant extracts and their active compounds (Sandeep Dhankhar, 2011). Plant-based chemicals have recently attracted significant attention due to their diverse applications. Medicinal plants comprise a group of species that contain a wide array of active ingredients, which can be used to treat various human and animal illnesses. They represent the most abundant source of bio-drugs on earth.

Recent advances in the discovery and understanding of phytochemicals have unveiled tremendous opportunities poised to revolutionize medicine, nutrition, and promote sustainability in both environmental and agricultural realms. Phytochemicals are naturally occurring compounds in plants, encompassing a diverse range of chemical classes such as flavonoids, alkaloids, terpenes, and phenolic compounds. While plants produce these phytochemicals primarily for their own defence against environmental stressors, their bioactivity when consumed by humans is nothing short of remarkable. The health benefits of phytochemicals are well-documented, showcasing their antioxidant, anti-inflammatory, and antimicrobial properties. The study of phytochemicals not only deepens our understanding of plant-based resources but also fosters innovative solutions to address pressing health and environmental challenges.

The exploration of phytochemical compounds—including alkaloids, flavonoids, phenols, saponins, tannins, glycosides, terpenoids, and coumarins, steroids are essential for fulfilling the pharmaceutical demand. Several plants have been studied for the quantification of secondary metabolites, including *Jatropha*, (Nwokocho et al., 2011), *Clerodendron colebrookianum*, *Zingiber cassumunar* (Majaw et al., 2009), *Spondias mombin* (Njoku et al., 2007). *Withania somnifera*

(Venkatasubbaiah and Savithramma, 2013), *Svensonia hyderabadensis* (Lingarao and Savithramma, 2011 & 2012) *Boswellia ovalifoliolata* (Savithramma et al 2010), *Syzygium alternifolium* (Yugandhar and Savithramma, 2017), *Terminalia pallida* (Ramamohan and Savithramma, 2019).

Here our work regarding to phytochemical analysis using with *Kydia calycina*, a member of the Malvaceae family known as Pulao, Boranga, or Pula, has been reported for its traditional uses as a medicinal plant in skin disease, hyperglycaemia, anti-hyperlipidemic, analgesic, anti-inflammatory, anticancer, antioxidant, antiulcer, antifungal, immunomodulatory, febrifuge, and wound healing (Praveen Kumar Goyal and Jeyabalan G 2020). The tribal of Rajasthan Maharashtra and Himachal Pradesh, *Kydia calycina* is reported to grow in a wide range of habitats, but mainly in arid regions for its nutritional importance (Dashahre AK et al., 2014). It is common in the deciduous forests of India and the sub-Himalayan tract (Bhattacharjee and Das, 1969). *Kydia calycina* is small tree and an evergreen tree growing. Number of plants was explored for secondary metabolites *K. calycina* is neglected so far hence the present study was undertaken to quantify the secondary metabolites from the leaves without harming of other parts of the plant.

MATERIAL AND METHODS

The *Kydia calycina* fresh leaves were collected from Srisailem area of Nallamala Forest, A.P. and these leaves brought into laboratory with sterile polythene bags. Initially, the leaves were thoroughly washed using running tap water to eliminate any surface dirt and contaminants. This was followed by rinsing with distilled water to ensure a clean starting material. After washing, moisture content and dust particles were removed using a sterile cotton cloth. The leaves were then cut into small pieces to facilitate the extraction process. Finally, this mass of material was shade-dried for 14 to 21 days to reduce moisture content before further use.

Chemicals

All the chemicals and reagents employed in this research were of analytical grade, ensuring the utmost accuracy and reliability in our experimental results. Utilizing analytical grade chemicals significantly reduces the risk of impurities.

Preliminary Phytochemical Screening

Phytochemical screening tests were conducted methanol (1:1) and

methanol crude extracts to identify the presence of various secondary metabolites. These tests comprehensively assessed alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, phenols, and glycosides, Saponins, adhering to the standard procedures of Nisha, et al., 2011; Bulama et al., 2014.

Quantitative Estimation of Primary and Secondary Metabolites

Based on the colour representations observed in qualitative phytochemical analysis, confidently quantified the prospective phytochemicals through rigorous quantitative analysis as per the established standard protocols. This included the precise measurement of proteins as outlined by Lowry et al. (1951), starch, McCready et al. (1950), sugars, DuBois (1956), and. Alkaloids Evans (2002), and flavonoids, Chang et al. (2002). tannins and phenols, Makkar et al. (1993).

RESULTS AND DISCUSSION

Preliminary phytochemical screening was conducted using selected polar and non-polar solvents to evaluate the presence of primary and secondary metabolites in the leaf material of the medicinal plant *Kydia calycina*. This observation assessed a total of 15 compounds, revealing that the methanolic extract yielded the highest number of phytochemicals (12), followed by the aqueous (11), ethanolic (11), and chloroform (9) extracts. In contrast, petroleum ether produced the lowest number of phyto-constituents (5), with acetone yielding slightly higher results (6). The specific findings are outlined in Table 1. The aqueous extract was notable for containing mucilage and gums, whereas fixed oils, fats, waxes, and lipophilic acids were exclusively extracted using petroleum ether. Amino acids were identified in both aqueous and methanolic extracts, with a higher concentration observed in the ethanolic extract. These results are accordance with earlier reports (Siva Prasad and Savithramma, 2016; Linga Rao and Savithramma, 2012).

Carbohydrates were present in the aqueous, ethanolic, and methanolic extracts. Sugars are currently the focus of extensive investigation. As they interact with diurnal changes, abiotic and biotic stresses, and hormone signaling, sugars are considered key components of a complex communication system necessary for coordinating metabolism with growth, development, and responses to environmental changes and stresses (Rolland et al., 2002, 2006). In particular, sugars such as the disaccharides sucrose and trehalose, raffinose family oligosaccharides, and fructans play a role in the management of reactive oxygen species (ROS) produced by plants in response to abiotic stresses. Well-known plant antioxidants include enzymatic scavengers such as superoxide dismutase, ascorbate peroxidase, and glutathione peroxidase, as well as non-enzymatic metabolites like ascorbate, glutathione, and α -tocopherol. Additionally, there is growing evidence that sugars themselves act as antioxidants, possessing ROS scavenging properties. Thus, sugars may be considered key components of an integrated cellular redox network.

Proteins, tannins, flavonoids, alkaloids, and glycosides were detected in all solvents, excluding petroleum ether, while terpenoids were confirmed in all solvents except chloroform. Proteins contribute to the structure and function of living cells. They occur both as independent units and in combination with lipids, nucleic acids, carbohydrates, and many other compounds. (Sabnis SD et al., 1990) Terpenoids are known for their analgesic and anti-inflammatory activities. Flavonoids have been reported to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular, and cytotoxic antitumour activities (Harborne JB and Williams CA, 2000). Phenolic compounds were predominantly detected in the aqueous, ethanolic, and methanolic extracts, with lesser presence in acetone. Steroids and terpenoids were successfully identified in ethanol, methanol, and acetone extracts, along with petroleum ether, while results from the other solvents were inconclusive. Notably, saponins were absent in both acetone and petroleum ether, affirming the integrity of the screening process. Based on the results, the highest concentration of phytochemicals was successfully isolated using methanol, ethanol, and aqueous solvents. In contrast, the other two solvents demonstrated a significantly lower yield of phyto-constituents. Therefore, it can be concluded that methanol, ethanol, and aqueous extracts of the plant are excellent sources for obtaining a greater number of phytochemicals. Anthocyanins help the human immune system function more efficiently, offering protection against viral infections. The underlying mechanisms are somewhat complex—certain types of anthocyanins

may directly reduce the infectivity of influenza viruses by decreasing their ability to enter human cells, limiting their release from infected cells, or by exerting a direct viricidal effect (Liu ALet al., 2009). Glycosides, flavonoids and alkaloids have hypoglycaemic activities (Oliver B, Ethnopharmacol J, 1980).

The analytical results for the quantitative estimation of different phytochemicals from leaf part of *Kydia calycina* clearly reveal a remarkable specificity and a rich diversity of metabolites. The quantitative analysis of primary metabolites unequivocally demonstrates the presence of a wide range of chemical constituents in the leaf of the plant, underscoring its significant potential for various applications based on these unique phytochemical profiles.

The analysis of *Kydia calycina* leaves reveals notable concentrations of primary and secondary metabolites, highlighting their potential value. Among the primary metabolites, starch (93.187 mg/gdw) and sugars (51.473 mg/gdw) were significantly predominant, outpacing proteins (21.093 mg/gdw) and lipids (0.293 mg/gdw). In terms of secondary metabolites, tannins exhibited the highest concentration at 61.343 mg/gdw. Tannins are widely distributed in nearly all plant foods (Serrano J et al., 2009). Remedies containing tannins are used as anti-helminthic (Ketzi JK et al., 2006), antioxidants (Koleckar V et al., 2008) antimicrobial agents, and antiviral agents, and phenols recorded at 14.253 mg/gdw. Numerous reports indicate that the presence of phenolics in foods is particularly important for oxidative stability and antimicrobial protection. (Carbanaro M, 2002). Phenols are ubiquitous secondary metabolites in plants and constitute a large group of biologically active compounds. To date, more than 8,000 phenolic compounds have been identified in plants. These phenolic compounds exhibit a wide range of biochemical activities, including antioxidant, anti-mutagenic, and anti-carcinogenic effects, as well as the ability to modulate gene expression. Although flavonoids were present at a lower level of 1.679 mg/gdw, the overall profile of these metabolites indicates the substantial biochemical potential of *Kydia calycina* leaves for various applications (Table 2). Flavonoids are secondary metabolites that are widely distributed in plants, with more than 6,000 different flavonoids identified to date. (Harborne JB and Williams CA, 2002). They are a group of polyphenolic compounds known for several important biological properties, including free radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action. (Frankel E, 1995) These properties are vital in combating free radicals that can damage human cells. (Cook NC, Samman S, 1996) Numerous epidemiological studies have confirmed a significant relationship between a high dietary intake of flavonoids and a reduction in cardiovascular and carcinogenic risks. Tannins are widely distributed in nearly all plant foods (Serrano J et al., 2009). Remedies containing tannins are used as anti-helminthic (Ketzi JK et al., 2006), antioxidants (Koleckar V et al., 2008) antimicrobial agents, and antiviral agents. These kinds of results observed in Leaf, Stem, and Bark of *Gymnosporia senegalensis* Quantitative Phytochemical analysis (Jain D et al., 2024).

CONCLUSION

The present study has demonstrated that the leaf extract of *Kydia calycina* contains a variety of secondary metabolites, which possess several pharmacological properties, including significant antioxidant activity. Although these compounds have recognized industrial applications, their pharmacological potential has not been thoroughly investigated. The metabolites identified in *Kydia calycina* leaves can be isolated with relative ease, indicating a valuable opportunity for further research. Continued exploration is essential to assess the potential for developing novel pharmaceutical products based on these bioactive compounds found in *Kydia calycina* leaves.

Author Contribution

Practical design and manuscript preparation were supervised by Prof. N. Savithramma, Department of Botany, Sri Venkateswara University, Tirupati. The first and second authors, conducted fieldwork and laboratory work assisted by our research supervisor Prof. N. Savithramma throughout the research.

Conflict of Interest Statement

The authors declared that they have no interest of conflicts.

Table 1: Preliminary Phytochemical screening of leaves of *Kydia calycina*

S. No	Phyto-chemical Constituents	Aque-ous Extract	Etha-nolic Extract	Metha-nolic Extract	Ace-tone Extract	Chloro-form Extract	Pet. Ether Extract	Tot al

1	Carbo-hydrates	+++	+	+	—	—	—	03
2	Proteins	++	++	++	+	+	—	05
3	Mucilage / Gums	+++	±	±	—	±	—	01
4	Amino Acids	+	±	+	—	—	—	02
5	Saponins	++	+	+	—	+	—	04
6	Tannins	++	++	++	+	+	—	05
7	Phenolic Compounds	++	++	++	+	—	—	04
8	Flavonoids	+	++	++	++	+	—	05
9	Alkaloids	+	++	++	+	+	—	05
10	Glycosides	+	++	++	+	+	—	05
11	Terpenoids	+	++	+	++	—	+	05
12	Steroids	—	+	+	+	—	+	04
13	Triterpenes	±	++	++	+	±	+	04
14	Fixed Oils & Fats	—	±	±	—	—	++	01
15	Waxes / Lipophilic Compounds	—	—	—	—	—	++	01
Total		11	11	12		06	05	54

+++ = Strong presence

++ = Moderate presence

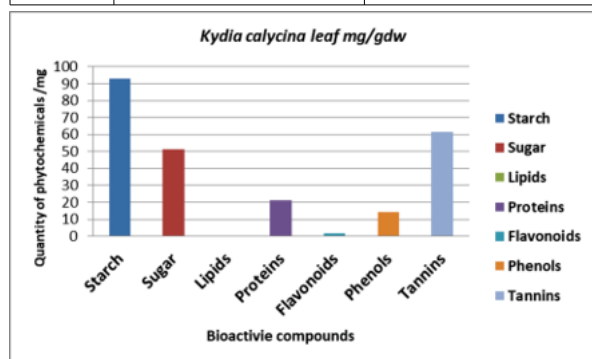
+ = Mild presence

± = Trace / doubtful

— = Absent

Table.2 Comparison Table of Quantitative Analysis of Phyto-constituents In Kydia Calycina

S. No	Phyto-constituents	Leaf (mg/gdw).
1	Starch	93.187 ±0.004
2	Sugar	51.473 ±7.105
3	Lipids	0.293± 0.002
4	Proteins	21.093±0.083
5	Flavonoids	1.679±0.016
6	Phenols	14.253± 0.012
7	Tannins	61.343 ±8.418

**Fig.1 Quantitative Estimation of Phyto-constituents in the Leaves of Kydia Calycina****REFERENCES**

- Bhattacharjee AK and Das AK: Phytochemical survey of few Mysore plants, economic botany, published by: Springer on behalf of New York Botanical Garden Press 1969; 23(3): 274-76.
- Bhukya BR: Evaluation of Preliminary phytochemical on various medicinal plants. International Journal of Allied Medical Sciences and Clinical Research 2014; 2(1): 79-82.
- Bulama JS, Dangoggo SM, Bwala YA, Abah JO. Phytochemicals and Antibacterial Evaluation of Root Bark Extract of Terminalia glaucescens. J App Pharm Sci. 2014;4(2):129-132. 13. Krishna VA, Rekha A, Sujathamma P. Studies on phytochemical analysis and antibacterial activity of leaf extracts of Terminalia tomentosa wight & arn. Int J Pharm Sci & Res. 2020;1(10):5142-47. doi: 10.13040/IJPSR.0975-8232.11(10).5142-47.
- Carbanaro M, Mattered M, Nicoli S, Bergamo P, Cappelloni M (2002). Modulation of antioxidant compounds in organic vs conventional fruit (peach, Prunus persica L., and pear, Pyrus communis L.). J Agric Food Chem; (50):5458-62.
- Cook NC, Samman S (1996). Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. Nutri Biochem (7):66-76.
- Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. Journal of Pharmaceutical Science, 55:225–276. <http://dx.doi.org/10.1002/jps.2600550302>.
- Frankel E. Nutritional benefits of flavonoids. International conference on food factors: Chemistry and cancer prevention. Japan: Hamamatsu; 1995. Abstracts, 6-2.
- Harborne JB, Williams CA, (2000). Phytochemistry, (55): 481-504.
- Harborne JB, Williams CA. Phytochemistry (2002), (55):481-504.
- Jain D, Meena, M, Janmeda P, Seth CS, Arora J (2024). Analysis of Quantitative

- Phytochemical Content and Antioxidant Activity of Leaf, Stem, and Bark of Gymnosporia senegalensis (Lam.) Loes. Plants (13); 1425. <https://doi.org/10.3390/plants13111425>.
- Ketzis JK, Vercruysse J, Stromberg BE, Larsen M, Athanasiadou S, Houdijk JG (2006). Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. Vet
- Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, et al.(2008). Condensed and hydrolysable tannins as antioxidants influencing the health. Mini Rev Med Chem;(8):436-47.
- Linga Rao M, Savithramma N (2012). Quantification of primary and secondary metabolites of Svensonia hyderbadensis– A rare medicinal plant. Int J Phar Phram Sci (4):519-21.
- Liu AL et al., Planta Med, 2009, 75: 337-9.
- Majaw S, Moirangthem J (2009). Qualitative and quantitative analysis of Clerodendron colebrookianum Walp. Leaves and Zingiber cassumunar Roxb. Rhizomes. Ethnobotanical leaflet (13):578-89.
- Nisha K, Darshana M, Madhu G, Bhupendra MK (2011) GC-MS analysis and antimicrobial activity of Psidium guajava (leaves) grown in Malva region of India. Int J Drug Dev Res 3(4):237–245.
- Njoku PC, Akumefula MI (2007). Phytochemical and nutrient evaluation of Spondias mombin leaves. Pak J Nutri (6):613-5.
- Nwokocha A, Blessing IO, Agabagwa, Okoli BE (2011). Comparative phytochemical screening of Jatropha L. species in the Niger Delta. Res J Phytochem (5):107-14.
- Oliver B, J Ethnopharmacol, 1980, 2: 119-127.
- Orhan, DD, Özçelik, B., Özgen, S., and Ergun, F. (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol. Res. 165, 496–504. doi: 10.1016/j.micres.2009.09.002
- Otaghviri AM, Yadav SR, Raina SN and Uniyal PL: Vegetational Wealth of Aravalli Ranges, Published by Scientific Publisher Jodhpur India 2015; 1:
- Parasitol;(139):321-35.
- Polturak, G., Misra, R. C., El-Demerdash, A., Owen, C., Steed, A., McDonald, H., et al. (2023). Discovery of isoflavone phytoalexins in wheat reveals an alternative route to isoflavonoid biosynthesis. Nat. Commun. 14, 6977. doi: 10.1038/s41467-023-42464-3.
- Rolland F, Baena-Gonzalez E, Sheen J (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu. Rev. Plant Biol. 57, 675–709. 10.1146/annurev.arplant.57.032905.105441 [DOI] [PubMed] [Google Scholar].
- Rolland F, Moore B, Sheen J. (2002). Sugar sensing and signaling in plants. Plant Cell 14(Suppl.), S185–S205. 10.1105/tpc.010455 [DOI] [PMC free article] [PubMed] [Google Scholar].
- Sabnis SD, Daniel M, A (1990). Phytochemical approach to economic Botany, Kalyani Publishers, New Delhi, (15); 65.
- Sandeep Dhankhar, Ramanjeet Kaur, S. Ruhil, M.Balhara, Seema Dhankhar and A.K. Chhillar, A review on Justicia adhatoda, a potential source of natural medicine, African journal of plant science, 2011;5(11):620-627.
- Serrano J, Puupponen-Pimia R, Dauer A, Aura AM, Saura-Calixto F (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. Mol Nutr Food Res; (53):310-29.
- Praveen Kumar Goyal and Jayabalan G (2020). A COMPREHENSIVE REVIEW ON PHARMACOLOGICAL PROPERTIES OF KYDIA CALYCINA, IJP, 2020; Vol. 7(7): 162-169.
- Dashahre AK, Navaneethan B, Bhutt P and Mahato S: Medicinal Plants of Sariska Tiger Reserve (Rajasthan) India. Journal of Medicinal Plants and Studies 2014; 2(2): 137-46.