Journal of Medicinal Herbs and Ethnomedicine 2024, 10: 7-11
doi: 10.25081/jmhe.2024.v10.8830
https://updatepublishing.com/journal/index.php/jmhe

Research Article

ISSN: 2455-0485

Qualitative and quantitative metabolomics of *Pamburus missionis* Swingle – a medicinal tree taxon

M. Sai Yaswanthi[1], P. N. Pallavi[2], N. Savithramma[1]*

[1]Department of Botany, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India, [2]Department of Biotechnology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India

ABSTRACT

Since ancient times, Plant based medicine is a well-known oldest form of healthcare to mankind. Even the commercial medications used for the treatment of various ailments today, contains a large proportion of plant derived chemical compounds. Hence chemical profiling of medicinal plants gained a key role to use them in pharmaceuticals as well as commercial industries. *Pamburus missionis* is one of the medicinal plants used in the Indian and Srilankan traditional medicine system to reduce kapha dosha. The ayurvedic physicians used its leaves to treat swelling, piles, fractures, fistula, puerperal diseases and as serpent venom. Therefore, the present study was undertaken to reveal the phytocompounds from various parts of this plant. Metabolomics revealed the presence of primary and secondary metabolites like carbohydrates, lipids, proteins, alkaloids, phenols, flavonoids, steroids etc. From the aqueous extract, Alkaloid content is almost same in leaf, stem and bark extracts with around 1.2 to 1.3 g (w/v%) and completely absent in the fruit. Phenolic content and steroids are notably high in leaf extract with 112.77±0.34 mg/g of extract and 17.91±0.26 mg respectively, where the flavonoid content in fruit extract with 44.69±0.30 mg/g of extract and the tannin content in bark extract with 62.37±1.75 mg/g of extract.

KEYWORDS: Metabolomics, *Pamburus missionis*, Medicinal plant, Phytochemicals

Received: February 19, 2024
Revised: May 09, 2024
Accepted: May 13, 2024
Published: May 23, 2024

***Corresponding author:**
N. Savithramma
E-mail: saiyaswanthi1997@gmail.com

INTRODUCTION

Plants are natural resources for the bioactive medicinal products. Secondary metabolites present in the plants, are the chief elements for treating various chronic as well as infectious diseases. These are non-nutritive chemicals well known for the plant's defence mechanism against diseases and herbivores (Mohan & Savithramma, 2019). Hence, thousands of plants have been used worldwide in the fields of medicine and pharma to prepare drugs with the properties like antimicrobial, antioxidant, anti-inflammatory, etc. (sharma *et al.*, 2020).

Rutaceae members have distinctive economic importance as fruits of genus *Citrus* (oranges, lime, lemons etc.,) and other species – *Aegle marmelos*, *Glycosmis pentaphylla*, etc., number of spices from *Zanthoxylum* species; ornamentally *Ptelea trifoliata* (Hop tree),*Skimmia* species; *Murraya koenigii* and *Ruta graveolens* are known for culinary and so many species are notable for medicinal importance because, the family is one of the richest sources for flavonoids, alkaloids, glycosides, phenols, etc (Parthasarathy *et al.*, 2008; Simpson, 2010; Panda *et al.*, 2019).

Pamburus missionis is a single species in this new genus from India (Swingle, 1916). Previously it was included in the genus *Limonia* (Wall.ex wight,1839), next in *Atalantia* (Oliv,1861). Due to numerous differences with *Atalantia*, a new genus name was created from the Singhalese word 'Pambaru', which means 'Hunter'. The plant is a small thorny citrus like - tropical dry evergreen tree, native to peninsular India and Sri Lanka (Pavithra *et al.*, 2009). It is found at degraded evergreen forests, scrub jungles and along the riverbanks from coastal plains up to 800-1000m. Medicinally in ancient days, the leaves are used for the treatment of swellings, fractures, piles and fistula while the decoction for phlegm and puerperal diseases (Jayaweera, 1982). Stem bark and flower are used for Cough, itches, mental delusion, arthritis, febrifuge (Jayasree *et al.*, 2015) and fruit yields fragrant oil which was used for chronic rheumatism and paralysis (Somasundaram, 1967). Lot of work on qualitative and quantitative screening of secondary metabolites had been carried out in the members of Rutaceae, whereas screening of metabolomics was not done so far in various parts of *P. missionis*. Hence the present research work was undertaken to find the metabolites from this plant.

MATERIALS AND METHODS

Plant Material

Pamburus missionis is a small tree with much branched stem (Figure 1) containing stout straight spines of 2-3 cm long (Figure 2). Leaves are alternate, oblong-obovate coriaceous with 5-10 cm long and 2-4 cm wide (Figure 3). Flowers are mostly tetramerous, white and hypogynous in axillary racemes. Fruit is globose and compressed berry, highly glandular (Figure 4). The leaves, stem, bark and fruits of the plant were collected from Mamanduru Forest, Karakambadi Rural, Tirupati, Andhra pradesh, India. The collected parts were washed under tap water, shade dried and ground into fine powder.

Extraction

The extracts were prepared with polar solvents like water, ethanol and methanol and non-polar solvents like chloroform and petroleum ether.

Aqueous extraction was made by dissolving 5 g of powder with 100 mL of distilled water and subjected to boil in a water bath for 30 min. Then it was filtered, and the filtrate was stored for further processes. Extraction through remaining solvents is done by macerating 5 g of each powder with 100 mL of each solvent for 24 hours and then filtered and stored (Peeriga & Banoth, 2016; De Silva *et al.*, 2017).

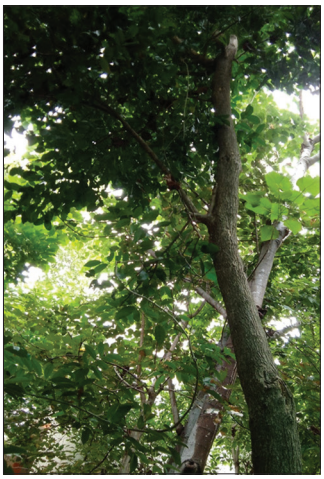

Figure 1: Tree of *Pamburus missionis*

Figure 2: Leaves of *Pamburus missionis*

Phytochemical Screening

With these extracts, different tests were performed to unveil the metabolites like Carbohydrates, lipids, proteins, alkaloids, phenols, flavonoids, saponins, terpenoids etc., by following the methods of Harbone (1973), Savithramma *et al.* (2011b), Shaikh and Patil (2020) and Sharma *et al.* (2020).

Quantification

Alkaloids, Flavonoids, Phenols, Tannins and Steroid compounds of the plant are quantified by following the methods of Okeke and Elekwa (2003), De Silva *et al.* (2017) and Sornapudi and Srivastava (2022).

RESULT

Screening tests for metabolomics revealed the presence of primary metabolic compounds like carbohydrates, proteins and lipids in the polar extracts of all parts except the reducing sugars which are found only in the fruit. In secondary metabolites, it was rich in alkaloids, flavonoids, phenols, glycosides, tannins, triterpenoids, coumarins and steroids. These compounds showed positive results in almost all solvents (Table 1).

When comparing, the aqueous extract of all parts showed a great number of compounds whereas the petroleum ether showed fewer compounds than methanol, ethanol and chloroform. Fruit has the highest count in almost all solvents, especially in aqueous and methanol, which is followed by leaves, stem and bark. Though fruit ranks first in secondary metabolites of all solvent extracts, alkaloids are completely absent in it, but saponins are found only in its polar solvent extracts. In aqueous extract, all the parts contain almost the same compounds aside saponins. In the methanolic and ethanolic extracts, only fruit

Figure 3: *Pamburus missionis* stem with thrones

showed almost all compounds which are found in its aqueous extract where the leaves, stem and bark did not expressed triterpenoids and coumarins. In the Chloroform extracts, tannins and steroids are present in all parts and alkaloids also, except in fruit. Flavonoids and triterpenoids are found only in fruit extract where the phenols are in leaves and stem. Coming to petroleum ether, only steroids are found to be in all parts along the fruit which showed positive result in flavonoids and triterpenoids also.

From the Table 1, Steroids are found to be in all solvent extracts of all parts. Phenols and flavonoids are found to be in polar solvents of all parts but completely absent in petroleum ether and chloroform extracts aside flavonoids in fruit and phenols in leaves and stem of chloroform extract. Glycosides are expressed in all polar solvent extracts but completely lacking in the non-polar solvent extracts. Tannins are present in all aqueous and chloroform extracts but absent in petroleum ether extract. In methanolic and ethanolic extracts, they were found to be in bark and fruit. Alkaloids are present in all solvent extracts aside petroleum ether of leaves, stem and bark. They were not expressed in any of the solvent extract of fruit. Triterpenoids are found only in the aqueous extract of all parts, but the fruit expresses, them in all solvents. Coumarins are also completely expressed in aqueous extracts of all parts. Saponins are only present in the polar solvents of the fruit. Anthraquinones and Anthocyanins are not expressed even in one solvent extract of a part.

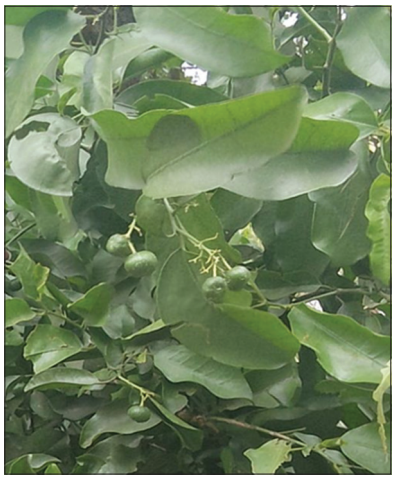

Figure 4: Fruit of *Pamburus missionis*

The estimation of metabolites from aqueous extract (Table 2 & Figure 5), the alkaloid content was slightly higher in stem (1.34±0.04) than leaves (1.31±0.01) and bark (1.23±0.01) mg/g. The total flavonoid content was calculated as 44.69±0.30 mg/g of extract in the fruit which stood high than Bark (24.12±1.76 mg/g), Stem (22.95±0.15 mg/g) and Leaves (19.35±0.62 mg/g). The total phenolic content was observed to be very high in the leaves with 112.77±0.34 mg/g when compared with Stem (68.31±1.65 mg/g), Bark (55.8±0.31 mg/g) and Fruit (38.7±0.84 mg/g). The total tannin content was calculated high with 62.37±1.75 mg/g of extract in bark continued by leaves with 61.83±0.26 mg/g, Stem and fruit with almost the same value 59.76±1.06 mg/g. The total steroid content was high in leaves with 17.91±0.74 mg/g followed by stem with 12.51±0.47 mg/g and fruit with 3.15±0.83 mg/g, where it was very low in bark with 0.72±0.15 mg/g.

DISCUSSION

From the results, the plant-parts extracts contain various phytocompounds which are marked for pharmaceutical applications. Predominantly, Rutaceae was renowed for phenols, flavonoids, terpenoids and especially coumarins. Almost the same results were found in *Syzygium alternifolium* (Yugandhar & Savithramma, 2017). Alkaloids are familiar for their anaesthetic, cardioprotective and anti-inflammatory properties (Heinrich *et al*., 2021). The Rutaceaec members containing alkaloids are *Citrus maxima*, *Murraya*, *Ruta Species*, etc (Panda *et al*., 2019; Sapkota *et al*., 2020). *Pamburus* also exhibited positive results for alkaloids in all parts aside from fruit. Some of the plants like *Dysophylla myosuroides*, *Talinum cuneifolium*, etc., (Savithramma *et al*., 2011a) are also lack of alkaloids as of fruit of *Pamburus*. Phenols and Flavonoids are almost present in all

Table 1: Metabolomics present in various solvent extracts of the plant parts

Compounds	Aqueous				Methonol				Ethanol				Petroleum Ether				chloroform			
Parts of plant	L	S	B	F	L	S	B	F	L	S	B	F	L	S	B	F	L	S	B	F
Carbohydrate	+	+	-	+	+	+	+	+	-	-	+	-	-	-	-	+	-	-	-	+
Proteins	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
Reducing Sugars	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Lipids	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-
Tannins	+	+	+	+	-	-	+	+	-	-	+	+	-	-	-	-	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Triterpenoids	+	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Coumarins	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

L=Leaves, S=Stem, B=Bark, F=Fruit, '+' indicates presence and '-' indicates absence

Table 2: Estimated values of the compounds (mg) in (g) of aqueous extracts

Compounds	Leaves (mg/g)	Stem (mg/g)	Bark (mg/g)	Fruit (mg/g)
Alkaloids	1.31±0.01	1.34±0.04	1.23±0.01	-
Flavonoids	19.35±0.62	22.95±0.15	24.12±1.76	44.69±0.30
Phenols	112.77±0.34	68.31±1.65	55.8±0.31	38.7±0.84
Steroids	17.91±0.74	12.51±0.47	0.72±0.15	3.15±0.83
Tannins	61.83±0.26	59.76±1.06	62.37±1.75	59.76±1.06

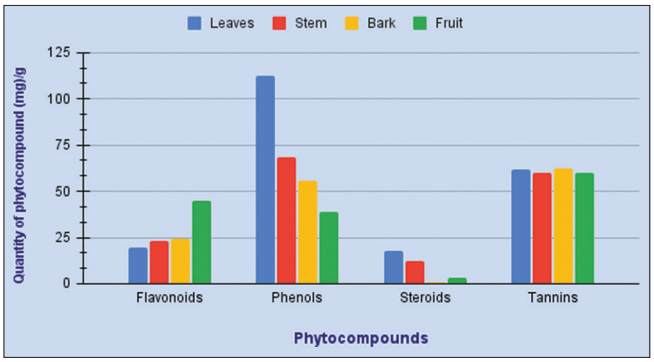

Figure 5: Quantity of Phytocompounds in different parts of the plant

medicinal plants. Notably, Phenols are well known for anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities (Lin *et al.*, 2016). Flavonoids are the widely distributed class of phenolic compounds which are found to be antimicrobial, antiulcer, antarthritic, anticancer etc. Tannins are analgesic and anti-inflammatory agents. They are more important for their astringic property i.e., faster healing of wounds and inflamed mucous membrane (Zhou *et al.*, 2024). The total phenolic content in rutaceae members is generally high as in *Zanthoxylum armatum* (Phuyal *et al.*, 2020), *Citrus limon*, *Citrus sinensis*, *Aegle marmelos*, etc., the *Pamburus* also followed the same. Coumarins are a class of lactones with benzopyrone structure used in perfumes, cosmetics and as medicinal candidates for drugs (Matos *et al.*, 2015). *Pamburus* contain coumarins like the species: *Quisqualis indica*, *Cleome viscosa*, *Polyalthia longifolia* (Savithramma *et al.*, 2011b; Savithramma *et al.*, 2012). Specific compounds of coumarins namely imperatorin, xanthyletin, xanthotoxin, scopoletin are isolated from the plant (Kumar *et al.*, 1994). Glycosides are important in medicine especially, cardiac glycosides which can be used as drugs in the treatment of congestive heart failure and cardiac arrhythmia. They are present in *Gomphrena serrata*, *Allamanda cathartica*, *Nyctanthes arbortristis*, *Chrysanthemum indicum*, etc (Savithramma *et al.*, 2012). Steroids usually show their effect on sex organs. *Nymphea* species are marked for steroids (Siva Prasad *et al.*, 2018) where we also got positive results. Anthraquinones and Anthocyanins are the pigment compounds belonging to the Flavonoid parent group. Anthraquinones are eminent for their purgative, antihyperlipidemic, immunoregulation effects where Anthocyanins are antioxidant in nature that are useful in the treatment of cancer, Alzheimer's and heart diseases (Zhou *et al.*, 2016; Wang *et al.*, 2021). But there are some plants like *Pamburus* which have no anthraquinones: In the leaves of *Abrus precatorius*, *Adhatoda vasica*, *Catharanthus roseus*, etc (Bhumi & Savithramma, 2014).

CONCLUSION

Based on the results, it is concluded that the plant is rich in alkaloids, coumarins, flavonoids, phenols, tannins and Steroids. Especially, Leaves contain high Phenolic content, Stem possess Alkaloid content, Bark with Tannin content and Fruit for Flavonoid. The medicinal importance and usage of a plant and its parts can be easily defined and understood with the help of metabolomics as it comes to know the types of metabolites present in it. Hence the present study was carried out on the plant *Pamburus missionis* Swingle to know its compounds such that it will be useful for further applications in pharmacological studies.

REFERENCES

Bhumi, G., & Savithramma, N. (2014). Screening of Pivotal medicinal plants for Qualitative and Quantitative phytochemical constituents. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(3), 63-65.

De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products, 5*(2), 29-32.

Harbone, J. B. (1973). *Phytochemical methods: A Guide to Modern techniques of Plant analysis.* London, UK: Chapman and Hill. https://doi.org/10.1007/978-94-009-5570-7.

Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids used as medicines: Structural Phytochemistry Meets Biodiversity -An Update and Forward Look. *Molecules, 26*(7), 1836. https://doi.org/10.3390/molecules26071836

Jayasree, S., Vijayakumar, N., Gomathi, K. S., & Helen, P. A. M. (2015). Study on Antimicrobial activity and Chemical profiling of *Pamburus missionis* (Wight) Swingle. *International Journal of Pharmacy and Pharmaceutical Research, 2*(3), 118-129.

Jayaweera, D. M. A. (1982). *Medicinal Plants (Indigenous and Exotic) Used in Ceylon*. Sri Lanka: National Science Council of Sri Lanka.

Kumar, V., Bulumulla, H. N. K., Wimalasiri, W. R., & Reisch, J. (1994). Coumarins and an indole alkaloid from *Pamburus missionis*. *Phytochemistry, 36*(4), 879-881. https://doi.org/10.1016/S0031-9422(00)90455-2

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and their importance in Human Nutrition and Mangement of Type 2 diabetes. *Molecules, 21*(10), 1374. https://doi.org/10.3390/molecules21101374

Matos, M. J., Santana, L., Uriarte, E., Abreu, O. A., Molina, E., & Yordi, E. G. (2015). Coumarins- an important class of Phytochemicals. In A. V. Rao & L. G. Rao (Eds.), *Phytochemicals-Isolation, Characterisation and Role in Human health* London, UK: IntechOpen Limited. https://doi.org/10.5772/59982

Mohan, P. R., & Savithramma, N. (2019). Screening of Phytochemicals and biosynthesis of silver nanoparticles from leaf, bark and fruits of medicinal tree species of *Terminalia pallida* Brandis an endemic to Seshachalam hill ranges. *The Pharma Innovation Journal, 8*(4), 408-416.

Okeke, C. U., & Elekwa, I. (2003). Phytochemical Study of the Extract of *Gongronema latifolium* Benth. *Journal of Health and Visual Science, 5*(1), 47-55.

Panda, M., Kumar, S., & Mahalik, G. (2019). An overview of medicinal plants of the family Rutaceae as a source of complementary therapeutics. *Journal of Biodiversity and Conservation, 3*(4), 13-17.

Parthasarathy, N., Selwyn, M. A., & Udayakumar, M. (2008). Tropical Dry Evergreen forests of Peninsular India: Ecology and Conservation Significance. *Tropical Conservation Science, 1*(2), 89-110. https://doi.org/10.1177/194008290800100203

Pavithra, P. S., Sreevidya, N., & Verma, R. S. (2009). Antibacterial activity and chemical composition of essential oil of *Pamburus missionis*. *Journal of Ethnopharmacology, 124*(1), 151-153. https://doi.org/10.1016/j.jep.2009.04.016

Peeriga, R., & Banoth, C. S. K. (2016). Pharmacognostical Investigation and Preliminary phytochemical screening of Leaves of *Pamburus missionis* S. *Journal of Comprehensive Pharmacy, 3*(3), 85-90.

Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of *Zanthoxylum armatum* DC. *The Scientific World Journal, 2020*, 8780704. https://doi.org/10.1155/2020/8780704

Sapkota, B., & Prakash, K. C., & Jain, V. (2020). Preliminary Phytochemical Screening and Quantitative Analysis of *Citrus maxima* (Brum.) Leaves Extract. *International journal of Research in Pharmacology and Pharmacotherapeutics, 9*(1), 100-106.

Savithramma, N., Rao, M. L., & Ankanna, S. (2012). Preliminary Phytochemical screening of some Important Medicinal plants. *International Journal of Ayurvedic and Herbal Medicine, 2*(1), 139-145.

Savithramma, N., Rao, M. L., & Prabha, B. (2011a). Phytochemical studies of *Dysophylla myosuroides* (Roth) Benth. In. Wall and *Talinum cuneifolium* (Vahl.) Willd. *Research Journal of Phytochemistry, 5*(3), 163-169.

Savithramma, N., Rao, M. L., & Suhrulatha, D. (2011b). Screening of Medicinal Plants for Secondary Metabolites. *Middle-East Journal of Scientific Research,* 8(3), 579-584.

Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies, 8*(2), 603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination. *Tribhuvan University Journal, 35*(2), 1-11. https://doi.org/10.3126/tuj.v35i2.36183

Simpson, M. G. (2010). Diversity and classification of Flowering plants: Eudicots. In M. G. Simpson (Eds.), *Plant Systematics* (pp. 275-448) Cambridge, US: Academic Press. https://doi.org/10.1016/B978-0-12-374380-0.50008-7

Siva Prasad, K., Sujatha, K., Suhrulatha, D., & Savithramma, N. (2018). Screening of *Nymphaea tetragona* Georgi, a traditional taxon fro bioactive metabolites. *Indian Journal of Applied Research, 8*(11), 22-24.

Somasundaram, T. R. (1967). *A Handbook on the Identification and Description of Trees, Shrubs and Some Important Herbs of the Forests of the Southern States: For the Use of the Southern Forest Rangers College.* Coimbatore, Tamil Nadu: Southern Forest Rangers College.

Sornapudi, S. D., & Srivastava, M. (2022). Quantitative estimation of Phytochemicals in different leaf extracts. *The Pharma Innovation Journal, 11*(9), 125-128.

Swingle, W. T. (1916). Pamburus, a new genus related to citrus, from India. *Journal of the Washington Academy of Sciences, 6*(11), 335-338.

Wang, D., Wang, X.-H., Yu, X., Cao, F., Cai, X., Chen, P., Li, M., Feng, Y., Li, H., & Wang, X., (2021). Pharmacokinetics of Anthraquinones from Medicinal Plants. *Frontiers in Pharmacology, 12*, 638993. https://doi.org/10.3389/fphar.2021.638993

Yugandhar, P., & Savithramma, N. (2017). Spectroscopic and chromatographic exploration of different phytochemical and mineral contents from Syzygium alternifolim (Wt.) Walp. an endemic, endangered medicinal tree taxon. *Journal of Applied Pharmaceutical Science, 7*(1), 73-85. https://doi.org/10.7324/JAPS.2017.70110

Zhou, Y., Zheng, J., Li, Y., Xu, D.-P., Li, S., Chen, Y.-M., & Li, H.-B. (2016). Natural Polyphenols for Prevention and Treatment of Cancer. *Nutrients, 8*(8), 515. https://doi.org/10.3390/nu8080515