A Preliminary Qualitative and Quantitative Phytochemical Screening of *Annona muricata* L. Leaf Using Various Solvent Extracts

Jerin Asha T[1*], Medo Merina R[2] and Bojaxa A Rosy[3]

[1]19223042262017, Ph.D Research Scholar, Department of Botany and Research Centre, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India.
[2] Assistant Professor, Department of Botany and Research Centre, Women's Christian College, Nagercoil, Tamil Nadu, India.
[3] Assistant Professor, Department of Botany and Research Centre, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India.
[*]Corresponding Author E-mail ID : aknaths.thileeban@gmail.com
Affiliated to Manonmanium Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu, India.

ABSTRACT

The main objective of the present study was to find out the phytochemicals present in aqueous, ethanol, chloroform, ethyl acetate and acetone extracts of *Annona muricata* L. (Soursop) by both qualitative and quantitative screening methods. In qualitative analysis various phytochemicals such as alkaloids, flavonoids, tannins, phenols, terpenoids, glycosides, saponins, steroids, carbohydrates, reducing sugars and proteins were present. In quantitative analysis total amount of available phytochemical constituents were quantified. The ethanol leaf extract showed maximum number of compounds, followed by ethyl acetate, chloroform and aqueous extracts. Least number of secondary metabolites present in acetone. The highest quantity of alkaloids shown in ethanolic leaf extract. The quantity of glycosides is remarkable in both ethanol and acetone. Among all phytochemicals present, terpenoids and reducing sugars showed the least quantity in all extracts. The results obtained during the study also highlights the value in the field of pharmacology to develop new drugs.

Keywords: Extracts, leaf, phytochemical screening, qualitative, quantitative, soursop,

INTRODUCTION

Plants represents a priceless tank of new bioactive molecules with various medicinal and pharmacological applications (Singh *et al*., 2014). Plants have been thus known to be a reservoir of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes. Numerous studies have been carried out to screen extracts from medicinal plants for the presence of novel compounds and to test their biological activities (Ahmedulla and Nayar,1999). Medicinal plants which contain phytochemicals are good for human health and for the prevention of diseases (Mlozi, 2022).

The phytochemical research that has been done based on the ethno-pharmacological information forms the effective approach in the discovery of new anti-infective agents from higher plants. It plays a vital role

against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these compounds do not have any side effects and also be considered as "man-friendly medicines".(Orhan *et al*.,2007).

Thus phytochemical screening is very important method of identifying bioactive compound that is useful in creating new drugs. These simple, cheap, sensitive, selective and rapid chemical tests to determine the presence of certain groups of compounds is an initial step to select plants for further phytochemical studies (Ibrahim, 2004).

Annona muricata L. produces natural compounds in its leaf, stem, bark, fruit and seeds. The leaves contain flavonoids, tannins, alkaloids, saponins, calcium, phosphorous, carbohydrate, vitamin A, B and C, phytosterol, calcium oxalate etc. The leaves shows active cytotoxicity against cancer cells, due to bioactive compounds called acetogenins. Its flavor possess a maximum of hundred and fourteen volatile compounds that is found to be responsible for the whole aroma profile, forty four esters, twenty five terpenes, ten alcohols, nine aldehydes and ketones, seven aromatic compounds, five hydrocarbons, three acids, three lactones and eight other miscellaneous compounds (Gajalakshmi *et al*., 2012). According to many scientists, up to ten thousand distinct phytochemicals may have the ability to influence conditions including cancer, stroke, and metabolic syndrome (Egbuna *et al*., 2019).

The present study of qualitative and quantitative phytochemical analysis like alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, tannins, glycosides, reducing sugars, carbohydrates and proteins in various solvent extracts is the preliminary work for the development of new drugs in order to treat various ailments.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh leaves of *Annona muricata* were collected from Thittuvilai (near Western Ghats), Kanyakumari District, Tamilnadu and were authenticated by experts in plant taxonomy. The leaves were separated from the stalk, washed and air-dried at room temperature (24ºc). Then crushed into fine powders and stored in air tight bottle and kept in a refrigerator.

Solvent extraction

The dried leaf sample (100 grams) was packed in three separate round bottom flask for sample extraction mixed using different solvents such as chloroform, acetone, ethyl acetate, ethanol and aqueous. The extraction was conducted by 25 ml of each solvent mixture for twenty four hours. At the end of the extraction, solvents were keep it in a water bath (at 50°c) and filled in sterile sample bottles and stored in refrigerator for further use. The procedure described by Selvakumar *et al*. (2019).

QUALITATIVE PHYTOCHEMICAL SCREENING

The extracts were tested by various standard methods to study the phytochemical constituents like as alkaloids, flavonoids, tannins, phenols, terpenoids, glycosides, saponins, steroids, carbohydrates, reducing sugars and proteins. Wanger's test for alkaloids, Shinodas test for flavonoids, Benedicts test for carbohydrates, Keller-Killani test for glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski test for terpenoids, Biuret test for proteins, Bromine water test for phenols,

Liebermann – Burchard test for steroids, Fehling's test for reducing sugars were determined.

QUANTITATIVE PHYTOCHEMICAL SCREENING

Various available phytochemicals in the extracts were quantified following the procedure described by Ingle *et al*.(2018) and phytochemicals were quantitatively analysed using spectrophotometer.

RESULT AND DISCUSSION

The qualitative and quantitative phytochemical analysis of *Annona muricata* L. leaf extract showed the presence of alkaloids, flavonoids, tannins, phenols, terpenoids, glycosides, saponins, steroids, carbohydrates, reducing sugars and proteins. The results are reported in Tab. 1-2 & Fig. 1-5. Chauhan & Mittu (2015) also studied the phytochemicals present in the leaves of *Annona muricata* and found the presence of carbohydrates, alkaloids, terpenoids, flavonoids, saponins, tannins and glycosides, etc. Among the eleven phytochemical constituents ethanol extract showed the presence of maximum number of phytochemical constituents (nine) such as alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, carbohydrates, reducing sugars and proteins.

In the present study phenols and steroids were not present but it was contrary with Hasmila *et al*.(2019) where phenols and steroids was observed. That may be due to the area of collection, abiotic stress, environmental factors and the deposition of heavy metals which influence the availability of phenols in plants (Pratyusha, 2022).

Ethyl acetate showed the presence of second highest constitutes (eight elements). Steroids, carbohydrates and proteins were not seen in ethyl acetate. This report was agreeing with the discovery of Oyedeji *et. al*., 2015. Seven phytochemical constituents were present in aqueous (alkaloids, flavonoids, tannins, glycosides, saponins, steroids and carbohydrates) and chloroform extracts (flavonoids, tannins, phenols, terpenoids, glycosides, saponins and reducing sugars). In our present study the aqueous leaf extract showed the absence of phenols, was agreed with the result of Roopashree *et al*. (2008) and Obasi *et al*. (2010).

Least number of phytochemical constituents present in acetone leaf extract, which showed the positivity of only three constituents such as flavonoids, tannins and glycosides. According to Naik & Sellapan (2019) also acetone showed the least number of phytochemical constituents in leaf extract.

The ethanolic leaf extract showed highest alkaloids content (640.04 ± 0.03), least amount in ethyl acetate leaf extracts and absent in chloroform and acetone. Alkaloids exhibit anti-proliferation, antibacterial, antiviral, insecticide and also showed effects on various types of cancers (Prasad *et al*.,2021; Mutakin *et al*., 2022). Most of the alkaloids would be more soluble in ethanol solvent. It makes them more soluble in the polar solvent, and reduces the solubility of acidic compounds reducing their concentration in the extract, making for a slightly easier clean-up. So alkaloids showed better effect in ethanol (Grinkevich & Safronich, 1983).

Flavonoids showed its maximum quantity in ethanol (260.03±0.004) and less in ethyl acetate (16±0). It promote overall health and potentially lower the risk of diabetes, cancers, cardiovascular and

neurodegenerative diseases (Paul *et al*., 2013). Tannins showed maximum quantity in acetone (324.01 ± 0.008) and less in ethyl acetate (44.06 ± 0.008). Tannins are used in the treatment of varicose, ulcers, haemorrhoids, minor burns as well as inflammation of gums (Ogbuagu *et al*., 2019). Phenol is absent in most of the leaf extracts such as aqueous, ethanol and acetone. The quantity of phenol is 130.02 ± 0.008 in ethyl acetate and 48.01 ± 0.008 in aqueous ectract.

Terpenoids showed trace amount in ethanol (7.006 ± 0.009), chloroform (8.0 ± 0) and ethyl acetate (3.0± 0.004) and absent in aqueous and acetone extracts. Terpenoids shows antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, anti-parasitic activities and memory enhancers (El-Omari *et al*., 2021). The quantity of Glycosides showed the second highest value (416.003 ± 0.004) and is prominent in both ethanol and acetone extracts and least in (22.006 ± 0.009). Glycosides are used as medicines for treating heart failure and certain irregular heartbeats (Paul *et al*., 2013).

The amount of saponin is higher in ethanol (200.676 ± 1.89) and chloroform showed less quantity (76.82 ± 0.01) and absent in acetone. Saponins used to reduce cholesterol and low density lipoprotein. It exhibit cytotoxic effect on cancer cells. The present study result relevant with Vimala *et al*. (2012) which said that ethanolic extract of *Annona* leaves has broad range of secondary metabolite saponins.

Steroids (160.01 ± 0.008) and proteins (137.05 ± 0.004) only showed the presence in aqueous and ethanol respectively. Steroids were used to reduce inflammation. This can ease symptoms of inflammatory conditions, such as arthritis, asthma and skin rashes (Alwan *et al*., 2020). The amount of carbohydrates showed moderate amount in aqueous (138.01 ± 0.008) and ethanol (160.01 ± 0.008) extracts and it is absent in chloroform, ethyl acetate and acetone. Trace amount of reducing sugars present in ethanol (4.03 ± 0.09), chloroform (10.10 ± 0.009) and ethyl acetate (8.036 ± 0.004). It was absent in aqueous and acetone.

The highest amount of alkaloids, glycosides and flavonoids present in ethanolic leaf extract is similar to the study made by Nandhini *et al*.(2018). The determination of the identity of the bioactive compounds from the leaves of *Annona muricata* L. forms a primary platform for further phytochemical and pharmaceutical studies (Okuwute, 1989).

Table. 1 Qualitative Analysis of the phytochemicals in *Annona muricata* L. leaves

S.No	Phytochemical Constituents	Aqueous	Ethanol	Chloroform	Ethyl Acetate	Acetone
1	**Alkaloids**	++	+++	-	+	-
2	**Flavonoids**	+	+++	+	+	+++
3	**Tannins**	+	+++	+++	+	+++
4	**Phenols**	-	-	+	++	-
5	**Terpenoids**	-	+	+	+	-

6	**Glycosides**	+	+++	+	++	+++
7	**Saponins**	++	+++	+	++	-
8	**Steroids**	+++	-	-	-	-
9	**Carbohydrates**	++	++	-	-	-
10	**Reducing Sugars**	-	+	+	+	-
11	**Proteins**	-	++	-	-	-

Figure.1 Number of Phytochemicals in *Annona muricata* L. leaves

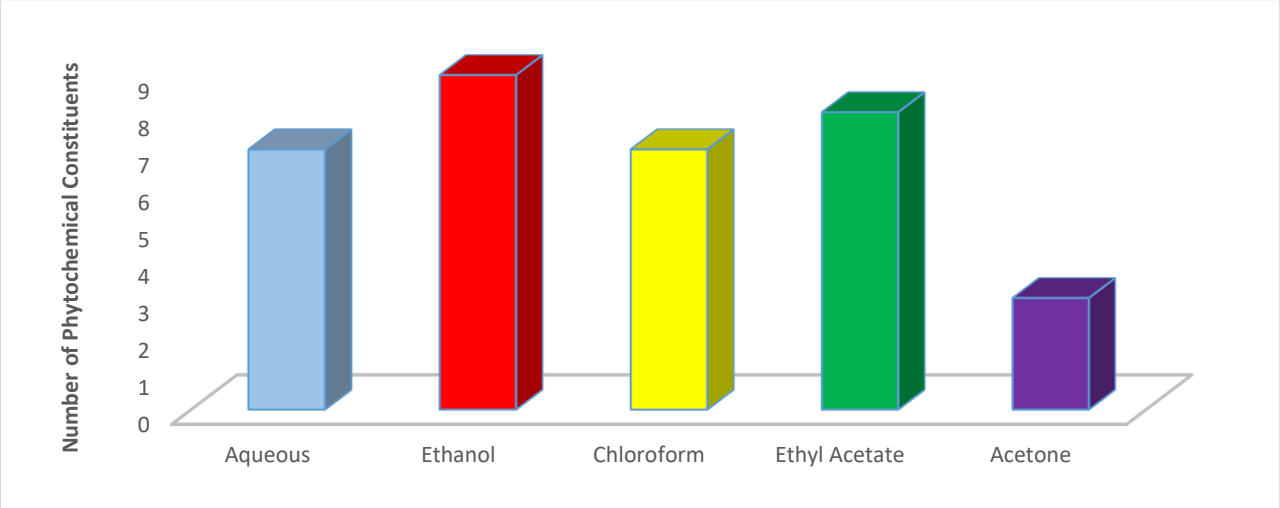


Table. 2 Quantitative Analysis of the phytochemicals in *Annona muricata* L. leaves

Phytochemical constituents	**Aqueous (µg/g)**	**Ethanol (µg/g)**	**Chloroform (µg/g)**	**Ethyl acetate (µg/g)**	**Acetone (µg/g)**
Alkaloids	134.004 ± 0.004	640.04±0.03	-	68.12 ± 0.009	-
Flavonoids	64.06 ± 0.04	260.03± 0.004	30.016 ± 0.004	16±0	209.02 ± 0.008
Tannins	72.066 ± 0.04	196.006 ± 0.04	224.033 ± 0.004	44.06 ±0.008	324.01 ± 008
Phenols	-	-	48.01±0.008	130.02 ± 0.008	-

Terpenoids	-	7.006 ± 0.009	8.0 ± 0	3.0 ± 0.004	-
Glycosides	56.506 ± 0.004	416.003 ± 0.004	22.006 ± 0.009	152.016 ± 0.004	416.003 ± 0.004
Saponins	116.006 ± 0.004	200.676 ± 1.89	76.82 ± 0.01	174.68 ± 0.94	-
Steroids	300.01 ± 0.004	-	-	-	-
Proteins	-	137.05 ± 0.004	-	-	-
Carbohydrates	138.01 ± 0.008	160.01 ± 0.008	-	-	-
Reducing sugars	-	4.03 ± 0.09	10.10 ± 0.009	8.036 ± 0.004	-

Figure. 2 Quantity of Phytochemicals present in *Annona muricata* L. leaves in various solvent extracts.

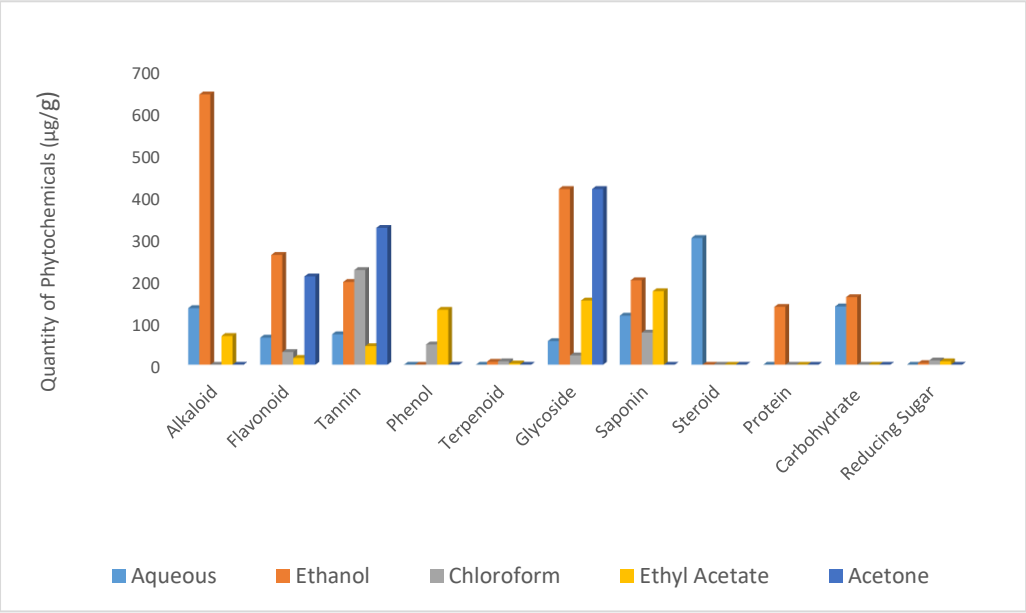


Figure. 3 Alkaloids present in *Annona muricata* L. leaves in various solvent extracts

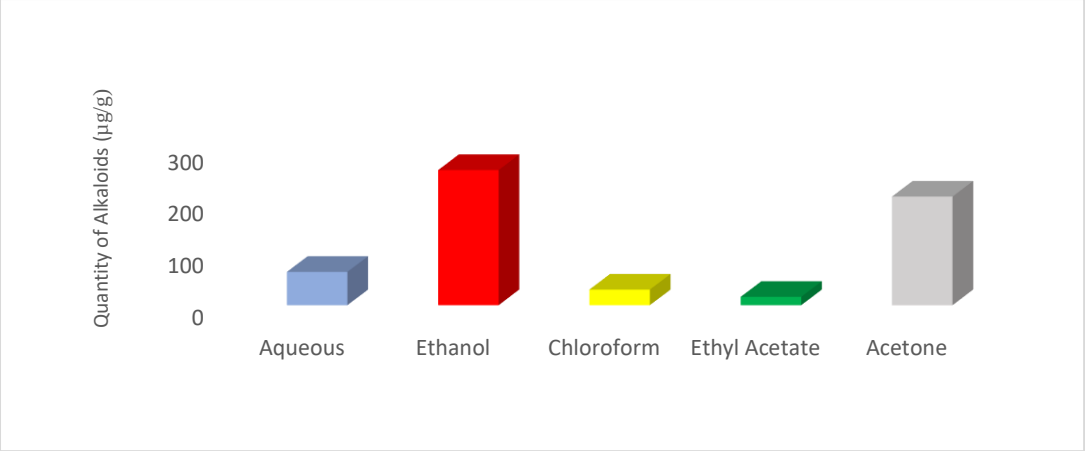


Figure. 4 Glycosides present in *Annona muricata* L. leaves in various solvent extracts

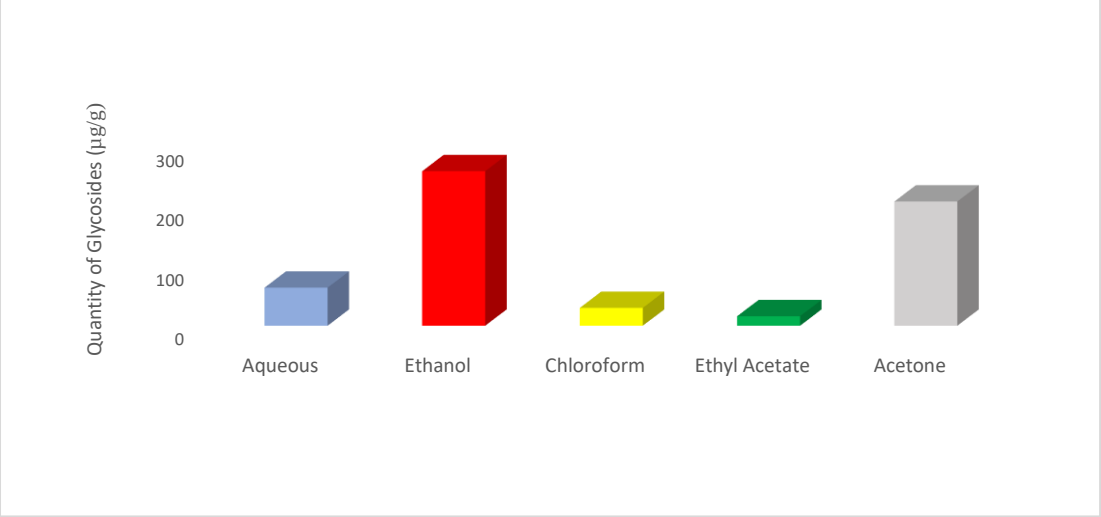
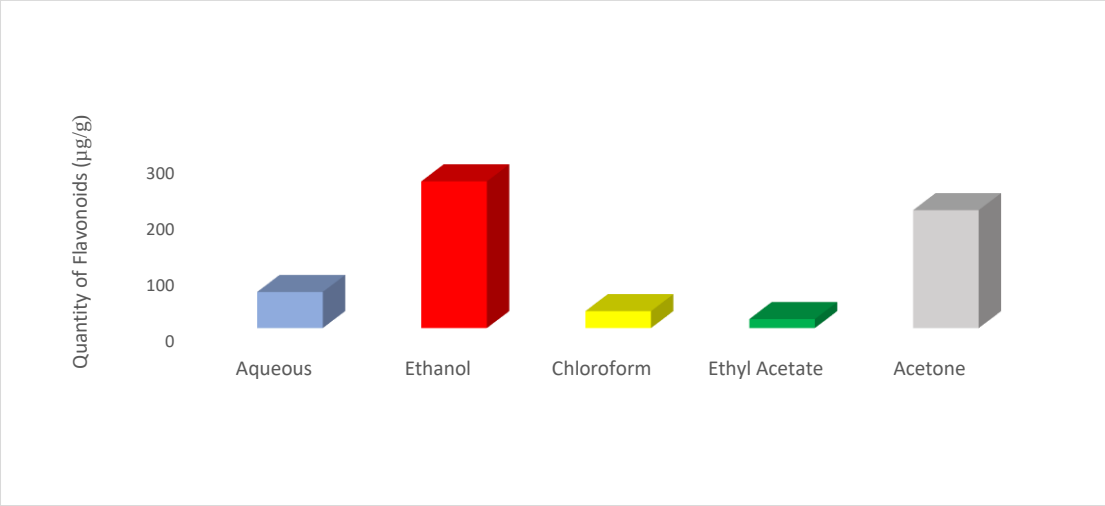


Figure. 5 Flavonoids present in *Annona muricata* L. leaves in various solvent extracts



CONCLUSION

The presence of phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, terpenoids, glycosides, saponins, steroids, carbohydrates, reducing sugars and proteins showed in various leaf extracts of *Annona muricata* is a promising source to enhance health. This preliminary study is important in understanding the discovery of new drugs. This study pave way for further pharmacological studies.

ACKNOWLEDGEMENT

The authors wish to thank Manonmanium Sundaranar University, Abishekapatti, Tirunelveli – 627012, Tamil Nadu, India.

REFERENCES

1. Ahemedulla, M & Nayar, MP 1999, Red Data Book of Indian Plants. Botanical Survey of India, vol.4.

2. Alwan, IQ, Lim, V, Samad, NA, Widyawati, T & Yusoff, NA 2020, 'Effect of *Annona muricata* L. on Metabolic Parameters in Diabetes Mellitus: A Systematic Review', Current Research in Nutrition and Food Science, ISSN: 2347-467X, Vol. 08, No.1, pp. 01-11.

3. Chauhan, A & Mittu, B 2015, 'Phyto-Chemical Screening and Anti-Listerial Activity of *Annona muricata* (L.) Leaf Extract', Journal of Chromatography Seperation Techniques, vol.6, no.3, doi.1000269.

4. Egbuna, C, Ifemeje, JC, Maduako, MC, Tijjani, H, Udedi, SC, Nwaka, AC & Ifemeje, MO 2019, Book: Phytochemistry - Fundamentals, Phytochemistry, Publisher: Apple Academic Press, USA, vol.1, ed.1,chap.15, pp.381-425.

5. El-Omari, N, Bakrim, S, Bakha, M, Lorenzo, JM, Rebezov, M, Shariati, MA, Aboulaghras, S, Balahbib, A, Khayrullin, M & Bouyahya, A 2021, 'Natural Bioactive Compounds Targeting Epigenetic Pathways in Cancer: A Review on Alkaloids, Terpenoids, Quinones, and Isothiocyanates', http://www.mdpi.com/journal/nutrients, vol.13, no.11, doi:org/10.3390/nu13113714.

6. Gajalakshmi, S, Vijayalakshmi, S & Rajeswari, DV 2012, 'Phytochemical and pharmacological properties of *Annona muricata*: A Review', International Journal of Pharmacy and Pharmaceutical Sciences, vol.4, no.2, pp.3-6.

7. Grinkevich, NI & Safronich, LN, 1983, Book: The chemical analysis of medicinal plants (in Russian), Publisher: Vysshaya Shkola, Moscow.

8. Hasmila, I, Natsir, H & Soekamto, NH 2019, 'Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.)', Journal of Physics: Conference, IOP Publishing, doi:10.1088/1742-6596/1341/3/032027.

9. Ibrahim, J 2004, 'Medicinal plant Research in Malaysia: Scientific Interests and Advances', Journal Sains Kesihatam Malaysia, vol.2, no.2, pp.22-46.

10. Ingle, KP, Deshmukh, AG, Padole, DA, Dudhare, MS, Moharil, MP & Khelukar, VC 2017, 'Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts', Journal of Pharmocognosy and Phyto chemistry, vol.6, no.1, pp.32-36.

11. Molzi, SH 2022, 'The role of natural products from medicinal plants against COVID -19: traditional medicine practice in

Tanzania', https://doi.org/10.1016/ jheliyon. 2022. e09739.

12. Mutakin, M, Fauziati, R, Fadhilah, F N, Zuhrotun, A, Amalia, R & Hadisaputri, Y E 2022, 'Pharmacological Activities of Soursop (*Annona muricata* L.) PubMed Central, Molecules, vol.27, no.4, doi:10.3390/molecules/27041201.

13. Nandhini, R, Pakutharivu, T, Rubalakshmi, G, Nirubama, K & Prabhakaran, S 2018, 'Phytochemical screening and antimicrobial activities of ever green medicinal plant *Annona muricata* - An edible vaccine', European Journal of Pharmaceutical and Medical Research, vol.5, no.7, pp.276-281.

14. Naik, AV & Sellappan, K 2019, 'Physicochemical and Phytochemical Analysis of Different Plant Parts of *Annona muricata* L. (Annonaceae)', Pharmaceutical Methods, Vol. 10,no. 2, pp. 70-78.

15. Obasi, NL, Egbuonu, ACC, Ukoha, PO & Eiikeme, PM 2010,'Comparitive phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods', African Journal of Pure and Applied Chemistry, vol.4, no.9, pp.206-212.

16. Ogbuagu, AS, Eric, SN & Obumselu, FO 2019,'Qualitative and Quantiative Phytochemical and Physicochemical Analyses on the Oil and Extracts from *Ricinodendron heudelotii*',Chemical & Pharmaceutical Research, vol.1, no.1, pp.1-7

17. Okwute, SK 1989, 'Unususal sources of antibacterial substances – A survey of some Nigerian plants', Nigeria Defence Academy Journal, vol.1, pp.133-141.

18. Orhan, I, Kupeli, E, Terzioglu, S, Yes & Ilada, E 2007,'A flavonoid with anti-inflammatory and anti-noci ceptive activity from the aerial part of *Calluna vulgaris* L.', Journal of Ethnopharmaclogy, vol.114, pp.32-37.

19. Oyedeji, O, Taiwo, FO, Ajayi, OS, Ayinde, F, Oziegbe, M, Oseghare, CO 2015, 'Biocidal and Phytochemical Analysis of Leaf Extracts of *Annona muricata* (Linn.)', International Journal of Sciences: Basic and Applied Research (IJSBAR), vol.24, no.7, pp.76-87.

20. Paul, J, Gnanam, R, Jayadeepa, R & Arul, L 2013, 'Anticancer activity on Graviola, an exciting medicinal plant extract vs various cancer cell lines and a detailed computational study on its potent anticancerous leads', Current Topics in Medical Chemistry, vol.13, no.14), pp. 1666-1673.

21. Prasad, SK, Pradeep, S, Shimavallu, C, Kollur, SP, Syed, A, Marraiki, N, Egbuna, C, Gaman, MA, Kosakowska, O, Cho, WC, Patrick-Iwuanyanwu, KC, Ortega Castro, J, Frau, J, Flores-Holguín, N, & Glossman-Mitnik, D 2021, 'Evaluation of *Annona muricata* Acetogenins as Potential Anti-SARS-CoV-2 Agents Through Computational Approaches', Frontiers in Chemistry, vol. 8, doi : 624716.

22. Pratyusha, S, 2022. Book: Plant Stress Physiology - Perspectives in Agriculture, Chapter: Phenolic Compounds in the Plant Development and Defense: An Overview, DOI:10.5772/intechopen.102873.

23. Roopashree, TS, Dang, R, Rani, SRH & Narendra, C 2008, 'Antibacterial activity of anti- psoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendulla officinalis*', International Journal of Applied Research and Natural Products, vol.1, no.3, pp.20-28.

24. Selvakumar, S, Vimalanban, S & Balakrishnan, G, 2019, 'Quantitative etermination of phytochemical constituents from *Anisomeles malabarica*', MOJ Bioequivalance & Bioavailability, vol.6, no.2, pp.19-21.

25. Singh, DR, Shrawan, S & Shajeeda, V 2014, 'Phytochemical composition antioxidant activity and sensory evaluation of potential underutilized fruit soursop (*Annona muricata* L.) in Bay island', Journal of Andaman Science Association, vol.19, no.1, pp.30-37.

26. Vimala, JR, Rose, AL & Raja, S 2012, 'A study on the phytochemical analysis and corrosion inhibition on mild steel by *Annona muricata* L. leaves extract 1N hydrochloric acid', Der chemical Sinica, vol.3, no.3, pp. 582-58.