Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

Phytochemical analysis and antimicrobial activity of Muntingia calabura

M. Sowjanya[1, *], L. Srinivasulu[2], Lakshmi B.K.M[3,] Ch. Venkatrayulu[4] and S.B.Sainath[5]

[1,4]Department of Marine Biology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India-524324
[2]SAI MARINE EXPORTS PVT ltd, Nellore, Andhra Pradesh, India-524324
[3]Department of Biochemistry, Andhra University, Visakhapatnam, Andhra Pradesh, India-530003
[5]Department of Biotechnology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India-524324

***Corresponding author**:

(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS:
Anti-Microbial activity, Muntingia Calabura and Phytochemicals.

Abstract

The purpose of this study was to demonstrate phytochemical screening and antimicrobial activity of Muntingia calabura plant extracts. Methanol was used to prepare the extracts from plant parts like the Leaf, Fruit and Root. The Methanolic extracts had comparable efficacy to that of the standard, against Staphylococcus aureus (MTCC-3160), Escherichia coli (MTCC- 443), and fungal cultures of Aspergillus niger (MTCC-961), Aspergillus flavus (MTCC-3396). Phenolics, tannins, saponins, and flavonoids were found in large quantities in Root extract. The current study demonstrates that the solvent extract of M.calabura contains bioactive compounds that are useful in medicine, which supports the use of plant species as traditional medicine to treat a variety of diseases. Similarly, the zone of inhibition with the fungus was greater in the root extract of Muntingia calabura. This demonstrates its antiseptic property, which may have been caused by the components present.

1. Introduction

Medicinal plants are major sources for many therapeutic solutions that are helpful to humans. Approximately 80% of people worldwide are using traditional medicine for primary health-care, this is due to 85% of plant extract is used as traditional medicine for many diseases. due to these interesting reasons research on phytomedicine has been increased since 20 years (Mahmood et al.,2014). In recent studies Muntingia calabura gained important status as medicinal plant. Muntingia calabura (Jamaica cherry) belong to family muntingiacae. The plant grows in tropics and subtropics worldwide which have an important role in medical field (Mahmood et al., 2014). So this plant is taken to as a novel discovery against different clinical applications, (i.e against many diseases) (Barza et al., 2002). The Jamaica cherry is used to reduce pain from gastric ulcer and also some other diseases. In earlier days this plant is used ayurvedic medicine against many diseases. The development of various types of diseases, both infectious and non-infectious. It is proven to have many pharmacological activities like anti-microbial activity, anti-fungal activity, anti-ulcer activity, anti-bacterial activity, and anti-diabetic activity (Mohini Upadhye et al., 2021).

The current study, on the other hand, was designed to evaluate the secondary metabolic compounds by using crude extracts of Muntingia calabura, based on the literature review and the lack of research on this plant.

2. Material and Methods

The Roots, flowers and fruits of Mutingia Calabura were collected at the Vikrama Simhapuri University campus, Nellore, Andhra Pradesh, India (Herbarium Voucher No- YVUH 5304) in the month of August, 2022. The roots, fruits and flowers of Mutingia calabura were cleaned, shade dried, and mechanically grinded. Finally, the coarse powders of selected plant materials were separated by sieving and stored in an air tight container for further use.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

Preparations of Extracts

Accurately weighed 50 gm of Mutingia calabura powdered roots, fruits and flowers were extracted with 250 ml of methanol by stirring at 50º C for 3 hr. The extracts were then filtered through What man filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure and temperature and stored in desiccator.

Evaluation of Phytochemicals

Estimation of total phenolics

The Folin-Ciocalteu method (Javanmardi et al.,2003) was used to estimate the amount of total phenolics in the extracts. 2003). Tests (200 µl) were brought into test tubes. 0.8 ml of sodium carbonate (7.5 percent) and one millilitre of Folin Ciocalteu reagent were added. After being mixed, the tubes were left to stand for 30 minutes. At 765 nm, absorption was measured. The standard gallic acid graph was used to calculate the total phenolic content, which was expressed as micrograms of gallic acid equivalents (GAE) per gram of extract.

Estimation of total flavonoids

A modified colorimetric method was used to estimate the total flavonoid content of the extract (Bao et al., 2005). A 5% NaNO2 solution and 1.0 ml of test extract were combined with 1 ml of distilled water and 75 ml. 75 l of a 10% AlCl3.H2O solution was added after 5 minutes. 0.5 ml of 1M sodium hydroxide was added after 5 minutes. The solution was thoroughly mixed and kept for 15 minutes. A UV-Visible spectrophotometer was utilized for the 510 nm measurement of the rise in absorbance. The standard quercetin calibration curve was used to calculate the total flavonoid content. Micrograms of quercetin equivalents (QE) per gram of extract were used to represent the outcomes.

Estimation of total tannins

The Folins-Ciocalteu method (1927) was used to estimate total tannins. Briefly, 0.1 milliliters of test extract, 6.5 milliliters of water, 0.5 milliliters of Folins-Ciocalteu reagent, and 1.5 milliliters of overnight standard solution containing 20% sodium carbonate were added and incubated for one hour. The absorbance of the sample was measured using a spectrophotometer at 725 n The results were expressed as micrograms of tannic acid equivalents per gram of extract, and the standard tannic acid calibration curve was used to calculate the total tannin content.

Estimation of Saponins

Obadoni and Ochuko's (2001) method was used. Conical flasks containing 25 g, 50 g, and 100 g of powder samples each received 100 ml of aqueous ethanol containing 20%. At approximately 55°C, the samples were heated for four hours with constant stirring in a hot water bath. After the mixture was filtered, another 200 milliliters of 20% ethanol were used to extract the residue. At around 90°C, the combined extracts were reduced to 40 ml in a water bath. The gather was moved into a 250 ml isolating pipe and 20 ml of diethyl ether was added and shaken vivaciously. The fluid layer was recuperated while the ether layer was disposed of. The process of purification was repeated. N-butanol-60 milliliters was added. Ten milliliters of aqueous sodium chloride containing five percent was used to wash the combined n-butanol extracts twice. In a water bath, the remaining solution was heated. The saponin content was calculated as a percentage after the samples were dried to a constant weight in an oven following evaporation.

Infrared (IR) Spectroscopy

Infrared spectrum of the compound was measured using Nicolet iS10 Fourier transform infrared (FTIR) spectrometer (Thermo Scientific, Massachusetts, United States) according to the manufacturer's protocol. IR spectra for the purified compounds were recorded on a Bruker series FTIR spectrometer using KBr pellets.

In-vitro Antimicrobial Activity

On nutrient agar medium, the disc diffusion technique and cup-plate method are used to stop the growth of bacteria in the study. The nutrient agar was weighed in a clean flask, autoclaved for 15 to 20 minutes at 121°C under 15 pounds of pressure, and then allowed to cool to room temperature. Before the nutrient agar media solidified, 100 l of test organisms were spread across the surface of the media in a sterile Petri dish. With the assistance of a sterile metal steel borer, wells were prepared. The wells were filled with plant extract at two different concentrations-20 g/ml and 40 g/ml-as well as a positive control and a negative control-DMSO at a concentration of 50 g/ml. For 14 hours, the plates were incubated aerobically at 37°C. According to (Bauer et al.,1966) the diameter of inhibition zones was measured in millimeters.

3. Results and Discussion

Evaluation of phytochemicals components

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

The high levels of phytochemicals found in the methanolic extracts of Mutingia calabura Fruits, Flowers and Roots suggest that these bioactive agents may have played a role in the folkloric use of the fractions in traditional medicine. The tests for flavonoids, phenols, tannins and saponins were performed with flowers, fruits and root powders. The results were recorded with the positive reports of certain phytochemicals.

Phenols

Phenolic acids and flavonoids are the most persistent group of plant phenolics that play significant role in plants, for human health and function as reducing agents and as free radicle scavengers (Mamta et al., 2012). The phenolic content present in the Muntingia Calabura roots was 130.66 ± 4.08mg of gallic acid equivalents g-1 of extract whereas for flowers120.66 ± 4.08mg, of gallic acid equivalents g^{-1} of extract and for fruits 110.66 ± 4.08mg of gallic acid equivalents/gm of extract. The levels of phytochemical constituents of analysis mentioned were at 100 mg/ml. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. When compared to the above result, root has the higest total phenolics content in Muntingia Calabura.

Flavonoids

Flavonoids act as signal molecules to take preventive measures in order to save them from pathogenic microbial attacks (Shirley, 1998). Recent studies have reaffirmed the link between flavonoids and plant architecture by showing that flavonoid-defective mutants display a wide range of alterations to root and shoot development (Buer et al., 2009). Flavonoids have recently been implicated in the anti-venom protease activity of some Nigerian tropical plants (Ibrahim et al., 2011). The Flavonoids content present in the Muntingia Calabura roots was 219.59 ± 5.50mg of gallic acid equivalents g-1 of extract whereas for flowers 175.66 ± 5.50mg, of gallic acid equivalents g^{-1} of extract and for fruits 200.67 ± 5.50mg of gallic acid equivalents/gm of extract. The levels of phytochemical constituents of analysis mentioned were at 100 mg/ml. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. When compared to the above result, root has the higest total flavonoids content in Muntingia Calabura.

Tannins

Tannins are reported to exhibit antiviral, antibacterial and antitumor activities and also used as diuretic (Aiyelaagbe and Osamudiamen 2009). Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues and also used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). The Tannins content present in the Muntingia Calabura roots was 13.16 ± 1.04mg of gallic acid equivalents g-1 of extract whereas for flowers17.16 ± 1.04mg, of gallic acid equivalents g^{-1} of extract and for fruits 10.16 ± 1.04mg of gallic acid equivalents/gm of extract. The levels of phytochemical constituents of analysis mentioned were at 100 mg/ml. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. When compared to the above result, Flowers has the higest total aTannins content in Muntingia Calabura.

Saponins

Saponins have been known to possess both beneficial and deleterious properties depending on its concentration in the sample (Seigler, 1998). Seigler (1998) reported that saponins have anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of growth of cancer cells and cholesterol lowering activity. Saponins have been found to be potentially useful for the treatment of hyperglycaemia (Olaleye, 2007). Saponins inhibit Na^+ efflux by the lockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+- Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} influx through this anti porter strengthens the contractions of heart muscle (Schneider and Woliling, 2004).The Saponins content present in the Muntingia Calabura roots was 18.645± 0.5mg of gallic acid equivalents g-1 of extract whereas for flowers 12.6 ± 0.5mg, of gallic acid equivalents g^{-1} of extract and for fruits 9.4 ± 0.5mg of gallic acid equivalents/gm of extract. The levels of phytochemical constituents of analysis mentioned were at 100 mg/ml. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. When compared to the above result, Roots has the higest total aTannins content in Muntingia Calabura.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

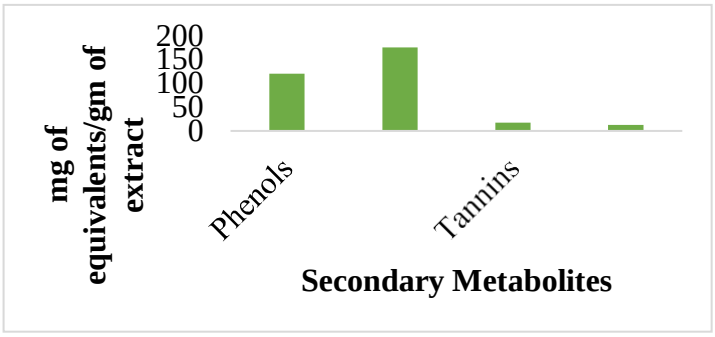

Fig.1. Quantitative analysis of flower extract of M.Calabura.

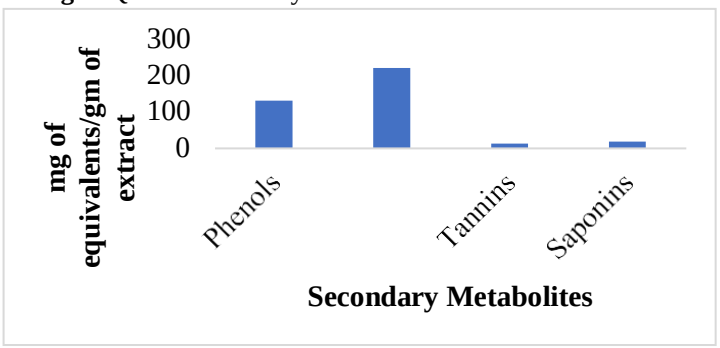

Fig. 2. Quantitative analysis of root extract of M.Calabura.

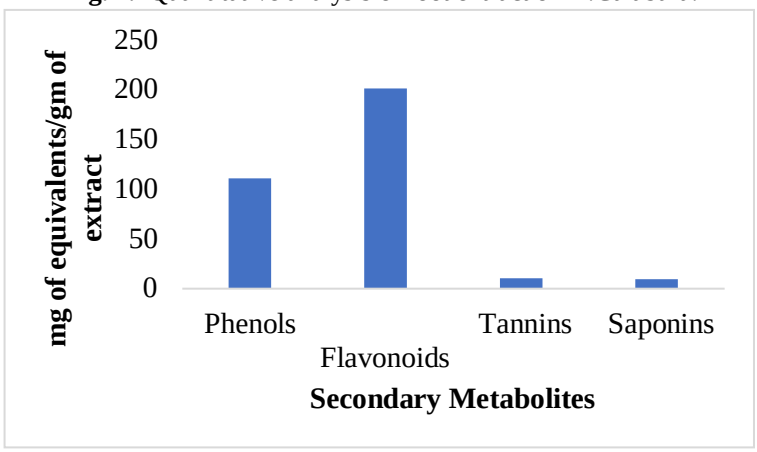

Fig.3. Quantitative analysis of fruit extract of M.Calabura.

Infra-Red Spectroscopy (FTIR) for Methanolic Flower Extract

These were recorded in KBr (AR) using Perkin Elmer Spectrum, FT-IR Spectrophotometer in the range 500-3500cm^{-1}. A pinch of sample (extract flower) and 50mg KBr thoroughly grounded in an agate mortar and the mixture was put on the pallet holder and pressure was applied through the hydraulic machine to make a thin film**.** IR spectrum of the isolated compound registered a broad band at 2739.69 cm-1 and 1500 stretching band. This indicates the presence of OH group and carbonyl (C=O) group. The spectrum also shows peak at 1793.54 cm-1, which indicates the presence of C=C stretching, C-O stretching and OH bending. The aliphatic and aromatic CH stretching is 2058.24 and 2444.84cm-1.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

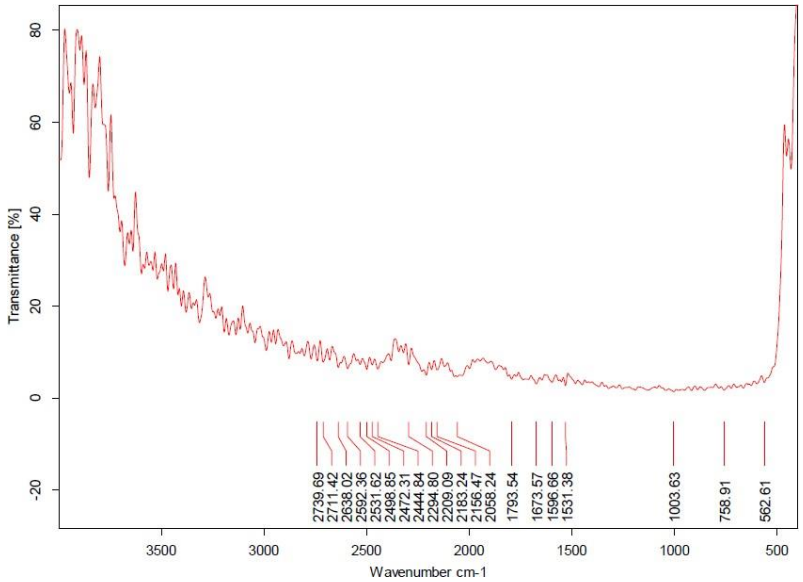

Fig.4. Infra-Red Spectroscopy (FTIR) for Methanolic Flower Extract.

Infra-Red Spectroscopy (FTIR) for Methanolic Root Extract

These were recorded in KBr (AR) using Perkin Elmer Spectrum, FT-IR Spectrophotometer in the range 500-3500cm-1. A pinch of sample (extract root) and 50mg KBr thoroughly grounded in an agate mortar and the mixture was put on the pallet holder and pressure was applied through the hydraulic machine to make a thin film. IR spectrum of the isolated compound registered a broad band at 3537.01 cm-1 and 1650 stretching band. This indicates the presence of OH group and carbonyl (C=O) group. The spectrum also shows peak at 2973.14 cm-1, which indicates the presence of C=C stretching, C-O stretching and OH bending. The aliphatic and aromatic CH stretching is 3458.95.

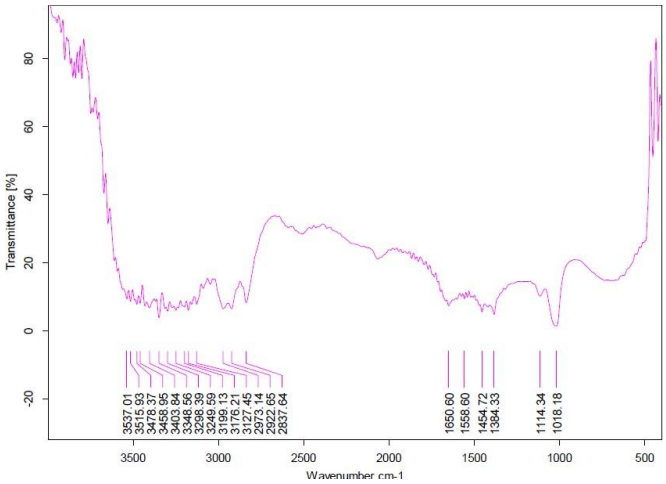

Fig.5. Infra-Red Spectroscopy (FTIR) for Methanolic Root Extract.

Antimicrobial Activity of Methanolic Extract - Flower, Root & Fruit of Muntingia Calabura

The antimicrobial activity of the different parts of the plant extracts were evaluated with the aim of discovering novel bioactive compounds of biomedical importance. The results of antibacterial and antifungal activities of purified compound were presented in Tables 1 and 8. Theantibacterial activity of Fruit, Flower and Root extracts showed

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

maximum zone of inhibition (15.3±53mm), (36.5±0.45mm), (29.4±0.56mm) against Escherichia coli (MTCC-443) and at the concentration of 800µg/mL. The antifungal activity of different plant extracts was evaluated by using different fungi. The highest antifungal activity flower extract (21.6±0.55mm) diameter of zone of inhibition against A. niger at the concentration of 800µg/mL, followed by fruit extract exhibit maximum zone of inhibition(19.2±0.50) against A. niger at the concentration of 800µg/mL, root extract exhibits maximum zone of inhibition (10.6±0.55) against A. niger at the concentration of 800µg/mL. When compared to the all theextracts, flower extract showed maximum antimicrobial activity.

The methanolic fruit extract of F. auriculata having capacity to control these bacterial infections. The antibacterial property was claimed to be conferred by phytochemicals present in the plant (Rekha at el., 2014). Our results support this view as methanol extracts had comparatively more inhibition action than aqueous extracts (Hugo et al., 2005). Methanolic extract of stem bark of F. auriculata shown the maximum zone of inhibition against E. coli and hexane leaf extract shown the maximum inhibition zone against S. aureus (Gaire et al., 2011). Imran and co-workers (2014) have demonstrated that the extracts and fractions of stem, root and leaves exhibited considerable antimicrobial activity against four bacterial P. aerugonisa locally isolated, E. coli ATCC 25922, Bacillus subtilis JS 2004, Bacillus cereus locally isolated) and two fungal strains 358 (Aspergillus niger and Candida albicans). Similarly, the methanolic extracts of stem, roots and leaves of F. benjamina were reported to have antibacterial activity. The presence of phenolic compounds and flavonoids mainly contribute the antibacterial activity against different bacterial strains (Imran et al., 2014).

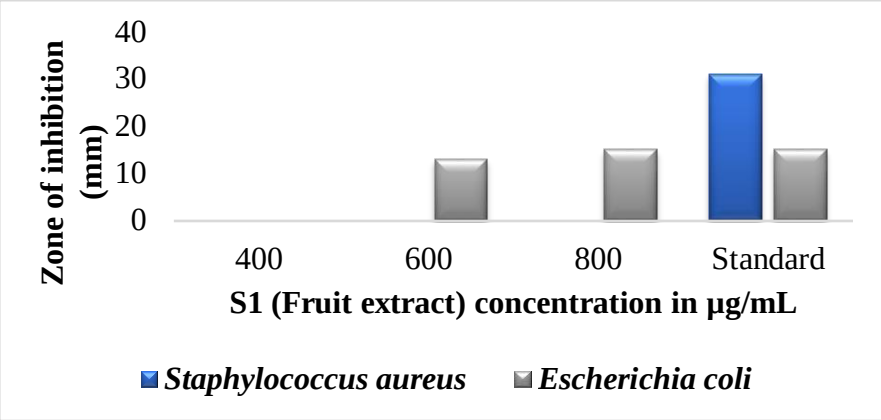

Fig.6. Antibacterial activity of S1 (Fruit extract) against selected bacteria.

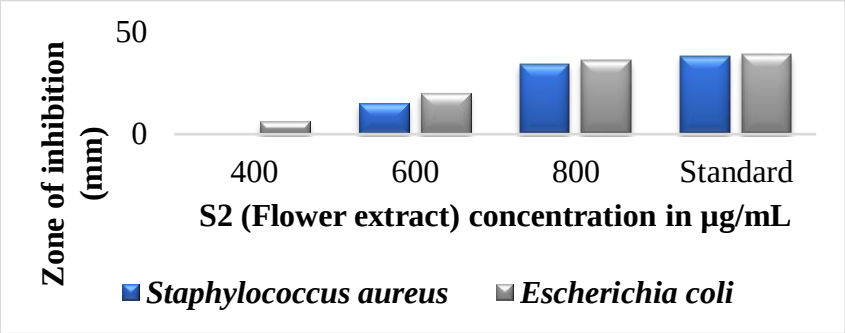

Fig.7. Antibacterial activity of S2 (Flower extract) against selected bacteria.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

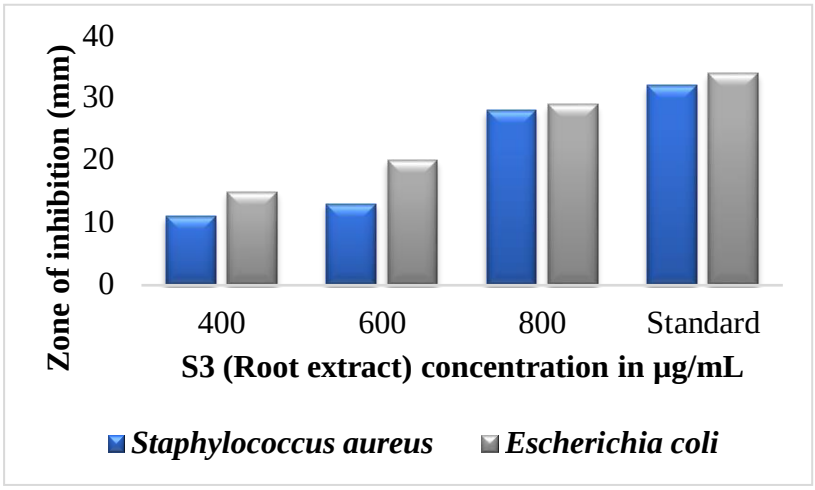

Fig.8. Antibacterial activity of S3 (Root extract) against selected bacteria.

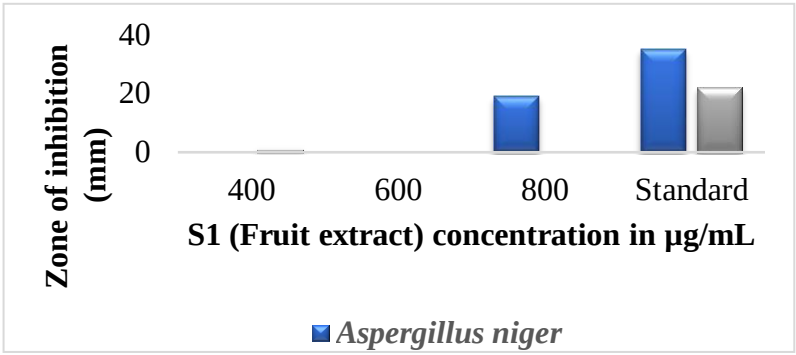

Fig.9. Antifungal activity of S1 (Fruit extract) against selected Fungi.

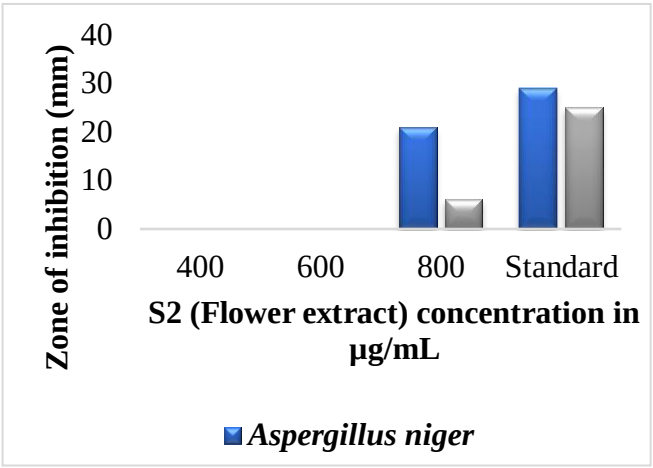

Fig.10. Antifungal activity of S2 (Flower extract) against selected Fungi.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

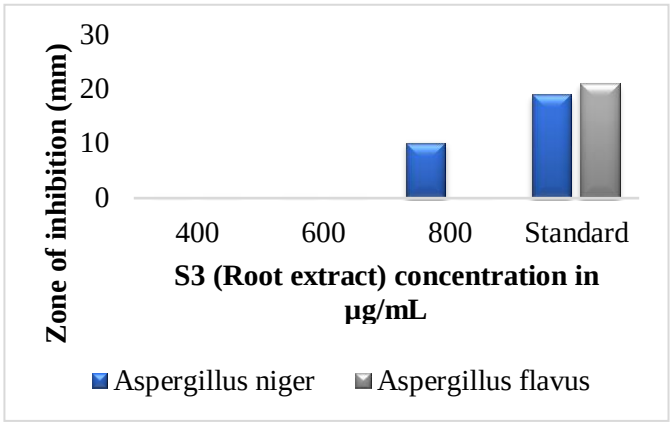

Fig.11. Antifungal activity of S3 (Root extract) against selected Fungi.

Statistical analysis

All the experiments were performed in triplicates and the results were expressed as mean ± standard deviation calculated using Microsoft Excel.

4. Conclusion

From the present study the quantitative analysis of secondary metabolites was analysed using standard methods. From the results obtained it can be concluded that the M.Calabura extract possesses significant secondary metabolites such as Flavonoids, Phenols, Tannins and Saponins. Quantitative screening revealed the presence of rich amount of Phenols (130.66 ± 4.08mg of gallic acid equivalents/gm of extract), Flavonoids (219.59 ± 5.50mg of gallic acid equivalents/gm of extract), and Saponins (18.645± 0.5mg of equivalents/gm of extract) in roots of Muntingia calabura whereas Tannins (17.16 ± 1.04mg of tannic acid equivalents/ gram of extract) the highest content is present in flowers. The identified secondary metabolites may also have properties such as antioxidant, antimicrobial, antidiabetic and anticancer etc. Further studies are needed to isolate, characterize and elucidate the structure of bioactive compounds.

5. Conflict of Interest: No

6. References

1. Barza, M.; Travers, K., Excess infections due to antimicrobial resistance: the "Attributable Fraction". Clin Infect Dis 2002, 34 Suppl 3, S126-30.
2. Bayer, C., 2003. Muntingiaceae. In Flowering Plants· Dicotyledons (pp. 315-319). Springer, Berlin, Heidelberg.
3. Chen, J.J., Lin, R.W., Duh, C.Y., Huang, H.Y. and Chen, I.S., 2004. Flavones and cytotoxic constituents from the stem bark of Muntingia calabura. Journal of the Chinese Chemical Society, 51(3), pp.665-670.
4. Javanmardi, J., Stushnoff, C., Locke, E. and Vivanco, J.M., 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food chemistry, 83(4), pp.547-550.
5. Kakuko Y, Fumiko A, Ariaki N, Hikaru O, Lucio L, Edith L. Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J Ethnopharmacology. 2005;97(2):293-9.
6. Kaneda, N., Pezzuto, J.M., Soejarto, D.D., Kinghorn, A.D., Farnsworth, N.R., Santisuk, T., Tuchinda, P., Udchachon, J. and Reutrakul, V., 1991. Plant anticancer agents, XLVIII. New cytotoxic flavonoids from Muntingia calabura roots. Journal of Natural Products, 54(1), pp.196-206.
7. Lamuela-Raventós, R.M., 2018. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. Measurement of antioxidant activity & capacity: recent trends and applications, pp.107-115.
8. Mahmood, N. D., N. L. M. Nasir, M. S. Rofiee, SF Mohd Tohid, S. M. Ching, L. K. Teh, M. Z. Salleh, and Z. A. Zakaria. "Muntingia calabura: A review of its traditional uses, chemical properties, and pharmacological observations." Pharmaceutical Biology 52, no. 12 (2014): 1598-1623.
9. Mandrik, C. A., & Bao, Y. (2005). Exploring the concept and measurement of general risk aversion. ACR North American Advances.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1041-1050 | ISSN:2251-6727

10. Marimuthu Krishnaveni and Ravi Dhanalakshmi. (2014). Qualitative and Quantitative Study of Phytochemicals in Muntingia calabura L. Leaf and Fruit. World Journal of Pharmaceutical Research, 3: 6.
11. Morton JF. (1987). Jamaica cherry. In: Morton JF, ed. Fruits of Warm Climates. Miami Florida, 2: 65-69.
12. Nasir, n.l.b.m., (2017). Antioxidant, anti-inflammatory and anti-proliferative effects of methanolic leaf extract of muntingia calabura l. On colon cancer.
13. Nirmala, M., Nisa, K.R. and Hulopi, F., 2022. Aromatherapy Soap Innovation from Clove Leaves as an Android-based Booklet Media in Applied Chemistry Learning. Journal Penelitian Pendidikan IPA, 8(2), pp.429-435.
14. Obadoni, B.O. and Ochuko, P.O., 2002. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of pure and applied sciences, 8(2), pp.203-208.
15. Okwu, D.E. and Josiah, C., 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology, 5(4), pp.357-361.
16. Prusti, A., Mishra, S.R., Sahoo, S. & Mishra, S.K. (2008), "Antimicrobial Activity of Some Indian Medical Plants". Ethno botanical Leaflets, 12: 227-230
17. Ramos, S., Oliveira, J., da Câmara, C.A., Castelar, I., Carvalho, A.F. and Lima-Filho, J.V., 2009. Antibacterial and cytotoxic properties of some plant crude extracts used in Northeastern folk medicine. Revista Brasileira de Farmacognosia, 19, pp.376-381.
18. Sani, M.H., Zakaria, Z.A., Balan, T., Teh, L.K. and Salleh, M.Z., 2012. Antinociceptive activity of methanol extract of Muntingia calabura leaves and the mechanisms of action involved. Evidence-Based Complementary and Alternative Medicine.
19. Sarojini S, Mounika BM. Jamaica cherry): An Overview. PharmaTutor. 2018;6(11):1-9.
20. Seigler, D.S., 1998. Phenylpropanoids. In Plant secondary metabolism (pp.106-129). Springer, Boston, MA.
21. Shen, Y., Jin, L., Xiao, P., Lu, Y. and Bao, J., 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. Journal of Cereal Science, 49(1), pp.106-111.
22. Shih CD, Chen JJ, Lee HH. Activation of nitric oxide signaling pathway mediates hypotensive effect of M. calabura L. (Tiliaceae) leaf extract. Am J Chin Med. 2006;37(6):1045-58.
23. Sibi G, Naveen R, Dhananjaya K, et al. (2012). Potential use of Muntingia calabura L. extracts against human and plant pathogens. Pharmacogn J 4:44-7.
24. Sirisha, N. and Rao, T.R., PHYSIOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF FIVE VARIETIES OF ARTOCARPUS PLANT EXTRACT.
25. Upadhye, M., Kuchekar, M., Pujari, R., Kadam, S. and Gunjal, P., 2021. Muntingia calabura: A comprehensive review. Journal of Pharmaceutical and Biological Sciences,9(2), p.81.
26. Woodson, R. E., Schery, R. W., & Smith, C. E. (1965). Flora of Panama. Part VI. Family 113. Elaeocarpaceae. Annals of the Missouri Botanical Garden, 52(4),487-495.
27. Yasunaka K, Abe F, Nagayama A, et al. (2005). Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J Ethnopharmacol 97:293–9.
28. Yusof MM, Teh LK, Zakaria ZA, Ahmat N. (2011). Antinociceptive activity of the fractionated extracts of Muntingia calabura. Planta Med. 77:PF21.
29. Zakaria ZA, Hazalin NAMN, Zaid SNHM, Ghani MA, Hassan MN, Gobalan HK, Sulaiman MR. (2007f). Antinociceptive, anti-inflammatory and antipyretic effects of Muntingia calabura aqueous extract in animal models. J Nat Med., 61:443-448.
30. Zakaria ZA, Jais AMM, Mastura M, Jusoh MSH, Mohamed AM, Jamil MNS, Rofiee MS, Sulaiman MR. (2007c). In vitro anti-staphylococcal activity of the extracts of several neglected plants in Malaysia. J Pharmacol., 3428-431.
31. Zakaria ZA, Kumar GH, Zaid SNH, Ghani MA, Hassan NAMN, Khamis MH, Devi GR, Sulaiman MR. (2007a). Analgesic and antipyretic actions of Muntingia calabura leaves chloroform extract in animal models. Orient pharm Exp Med., 7:34-40
32. Zakaria ZA, Mohamed AM, Jamil NSM, Rofiee MS, Hussain MK, Sulaiman MR, Teh LK, Salleh MZ. (2011). In vitro antiproliferative and antioxidant activities of the extracts of Muntingia

calabura leaves. Am J Chin Med., 12:280-292.

33. Zakaria ZA, Mustapha S, Sulaiman MR, Jais AMM and Fatimah CA. (2007e). The antinociceptive action of aqueous extract from Muntingia calabura leaves: The role of opioid receptors. Med Prin Pract., 16:130-136
34. Zakaria ZA, Somchit MN, Sulaiman MR, Jais AMM, Fatimah CA. (2008). Effects of various receptor antagonists, pH and enzymes on Muntingia calabura antinociception in mice. Res J Pharmacol., 2:31-37
35. Zakaria ZA, Sufian AS, Ramasamy K, Ahmat N, Sulaiman MR, Arifah AK, Zuraini A and Somchit MN. (2010). In vitro antimicrobial activity of Muntingia calabura extracts and fractions. Afr J Microbiol Res., 4: 304 -308.
36. Zakaria ZA, Sulaiman MR, Jais AMM, Somchit MZ, Jayaraman KV. (2006a). The antino-ciceptive activity of Muntingia calabura aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice. Fundam Clin Pharmacol., 20: 365 - 372.
37. Zakaria ZA. (2007b). Free radical scavenging activity of some plants available in Malaysia. Journal Pharmacology and Therapeutics, 6: 87 - 91
38. Zakaria ZA., Hassan MH, Aqmar, Ghani MA, Zaid MSNH, Sulaiman MR, Kumar GH, Fatimah CA. (2007d). Effects of various nonopoid receptor antagonist on the antinociceptive activity of Muntingia calabura extracts in mice. Methods Find Exp Clin Pharmacol., 29: 515 - 520.
39. Zakaria, Z. A., Sufian, A. S., Ramasamy, K., Ahmat, N., Sulaiman, M. R., Arifah, A. K.,& Somchit, M. N. (2010). In vitro antimicrobial activity of Muntingia calabura extracts and fractions. Afr J Microbiol Res, 4(4), 304-308.