EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 1
400-408
Year: 2022

ISOLATION AND CHARACTERIZATION OF LUTEOLIN 7 - GLUCOSIDE IN *MATHUKA NERIIFOLIA* (MOON) H.J. LAM. ETHANOL EXTRACT

Dr. Minu B. Pattasseril* and Dr. Sujith Abraham

Professor and Vice Principal, KMCT College of Pharmaceutical Sciences, Kallanthode, 673601, Kozhikode, Kerala.

***Corresponding Author: Dr. Minu B. Pattasseril**

Professor and Vice Principal, KMCT College of Pharmaceutical Sciences, Kallanthode, 673601, Kozhikode, Kerala.

Article Received on 17/11/2021 **Article Revised on 06/12/2021** **Article Accepted on 27/12/2021**

ABSTRACT

Herbal medicine is making a dramatic comeback as the side effect of synthetic medicine are daunting and the therapeutic approach is drifting towards substituent medicine. Luteolin is a naturally-occurring flavonoid, with potential anti-oxidant, anti-inflammatory, apoptosis-inducing and chemopreventive activities. Upon administration, luteolin scavenges free radicals, protects cells from reactive oxygen species (ROS)-induced damage and induces direct cell cycle arrest and apoptosis in tumor cells. This inhibits tumor cell proliferation and suppresses metastasis. Madhuca neriifolia (Moon) H.J.Lam. is an endangered plant species occurring in the Southern, Western Ghats of India. Flowers are used in the treatment of kidney complaints. Fruits are recommended in cases of rheumatism, biliousness, consumption, asthma and worm trouble. Oil from seeds is used to treat rheumatism and for improved growth of hair. Fractnation of ethanol extract of *Mathuka Neriifolia* (Moon) H.J. Lam. by column chromatography was done. During the column elution process, the fractions 260 - 300 has a single banding pattern which was confirmed by TLC study. Phyochemical screening showed the presence of Flavonoids, Phenolic and Tannins. Physical and chemical tests of isolated compound was conducted. By using IR, LC-MS, ^{1}H-NMR and ^{13}C-NMR spectra of the isolated compound was confirmed as Luteolin.

KEYWORDS: Luteolin 7 - glucoside, Mathuka Neriifolia (Moon) H.J. Lam., Phytochemical screening, flavonoid.

INTRODUCTION

The use of traditional medicines and medicinal plants as therapeutic agents for the maintenance of good health has been widely observed. Interest in medicinal plants as a re-emerging health aid has been fueled by the rising costs of prescription drugs. The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine and also less risk of side effects when compared to modern drugs.However, among the estimated 2,50,000 to 4,00,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically. This shows a need for planned activity guided phyto-pharmacological evaluation of herbal drugs, since most of the modern drugs has its natural product prototype. *Mathuka Neriifolia* (Moon) H.J. Lam. belongs to the family Sapotaceae. It is an endangered plant species occurring in the Southern, Western Ghats of India. Flowers are used in the treatment of kidney complaints. Fruits are recommended in cases of rheumatism, biliousness, consumption, asthma. It is an endemic species of Ceylon that claims to have medicinal uses like "The bark is a good remedy for itch, swellings, fractures and snakebite poisoning. The oil extracted from the seed is applied to swellings, rheumatism and other skin diseases. The Heartwood made into a paste is applied on the throat for glandular swellings in the neck and the throat. The oil is applied on wounds and sores caused by bears." and worm trouble. Oil from seeds is used to treat rheumatism and for improved growth of hair. The secondary metabolites, Flavonoids, a pigments that color most flowers, fruits, and seeds are widely distributed in plants with different metabolic functions. These polyphenolic compounds are ubiquitous group characterized by the flavan nucleus and available as a group of bioactive compounds in fruits, vegetables and plant-derived beverages. The basic structure of flavonoids is diphenylpropane skeleton, namely, two benzene rings (ring A and B – Fig. 1) linked by a three carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with benzenic A ring. So, its structure is indicated as C6-C3-C6. In most cases, B ring is attached to position 2 of C ring, but it can also bind in position 3 or 4; this, together with the structural features of the ring B and the patterns of glycosylation and hydroxylation of the three rings, makes the flavonoids one of the larger and more diversified groups of phytochemicals.

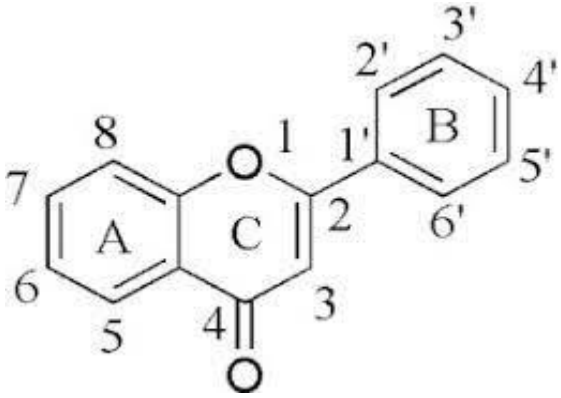


Fig 1.

Depending on the carbon of the C ring on which B ring is attached, and the degree of unsaturation and oxidation of the C ring, Flavonoids are sub classified into different types1. Those Flavonoids in which B ring is linked in position 3 of the ring C are called isoflavones; those in which B ring is linked in position 4, neoflavonoids, while those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring. These subgroups are: flavones, flavonols, flavanones, flavanonols, flavanols or catechins and anthocyanins. Finally, flavonoids with open C ring are called chalcones.

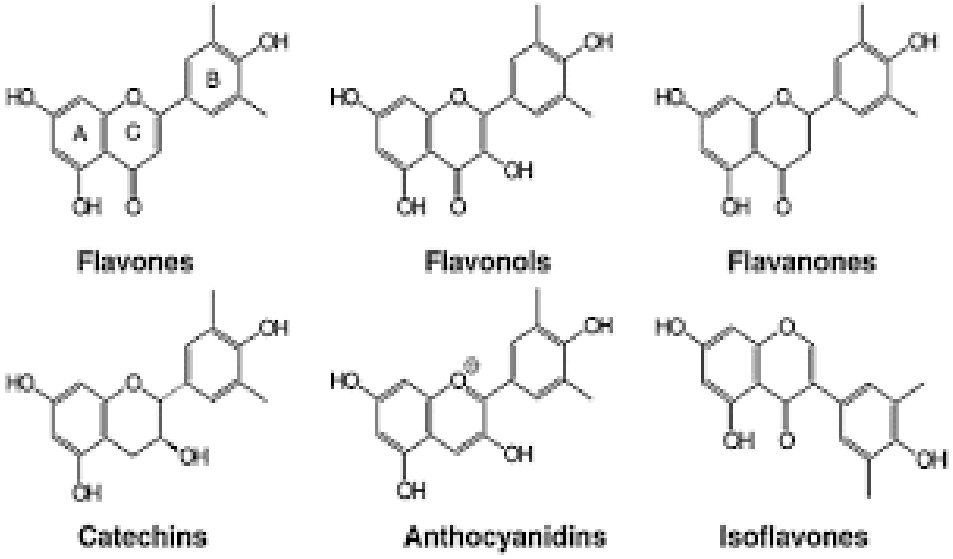


Fig 2.

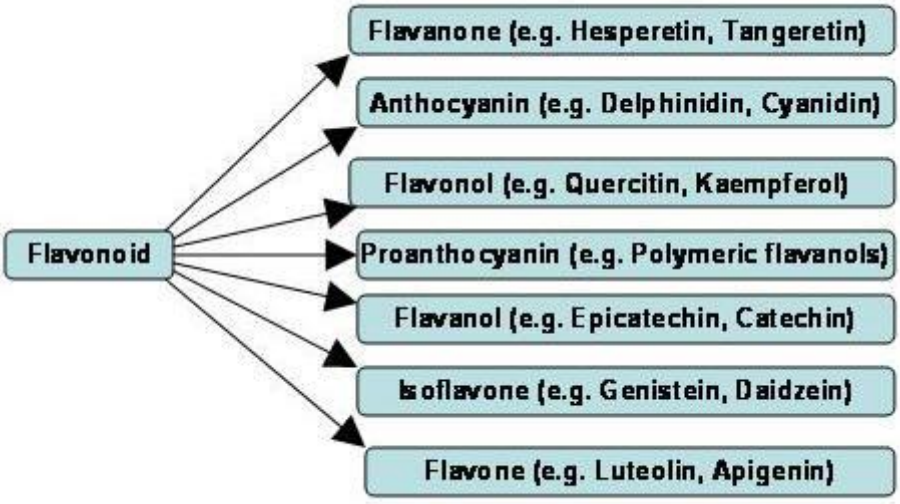


Fig 3.

Flavonoids are mainly divided into seven major groups (Fig.:3). One of the best described flavonoids, Luteolin is a member of this group. Luteolin is a naturally-occurring flavonoid, with potential anti-oxidant, anti-inflammatory, apoptosis-inducing and chemopreventive activities. Upon administration, luteolin scavenges free radicals, protects cells from reactive oxygen species (ROS)-induced damage and induces direct cell cycle arrest and apoptosis in tumor cells. This inhibits tumor cell proliferation and suppresses metastasis. The qualitative chemical tests on

the plant extracts will help to detect the various Phyto-constituents present. The spectroscopies are the primary method for determining the structure of compounds. By using IR, LC-MS, ^{1}H-NMR and ^{13}C-NMR spectra of the isolated compound will help confirm the structure of a compound.

MATERIALS AND METHODS

Preparation of extracts

The fresh plant parts of ***Mathuka Neriifolia*** (Moon) H.J. Lam. was shadow dried and powdered. Powdered material was passed through sieve No.60. Then extracted separately using hexane, petroleum ether, chloroform, ethyl acetate, ethanol and water by the Soxhlet extraction method. The hot percolation method was employed for water for 48 hrs. The extracts were concentrated using a rotary vacuum evaporator.

Qualitative phytochemical Screening

Various qualitative tests were performed on the various extracts of ***Mathuka Neriifolia*** (Moon) H.J. Lam. for the identification of phytoconstituents.

Detection of carbohydrates

500mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Molisch' s test

Molisch reagent: 10 g of alpha napthol was dissolved in 100ml of 95%methanol to prepare Molisch reagent.

To the extract two drops of molisch reagent was added and a few drops of conc. H_2SO_4 is added, formation of the purple - violet ring indicates the presence of carbohydrates.

Detection of Glycosides

Keller-Killiani test: Add glacial acetic acid to the extract and one drop of 5%$FeCl_3$ and con H_2SO_4 was added, formation of reddish brown color at the junction of two liquid layers and upper layer turned bluish green indicates the presence of glycosides.

Detection of Saponins

Foam test: 1 ml of extract was diluted to make up to 20ml with distilled water and slowly shacked in a graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

Detection of Alkaloids

To the residue add dil HCl. Shake well and filter. With the filtrate perform the following tests.

a.Dragendorff's test

Dragendroff's reagent: Reagent available from Sd fine chemicals, Mumbai.

To 2-3 ml filtrate adds few drops Dragendorff's reagent. Orange brown ppt is formed.

b. Wagner's test

Wagner's reagent: Reagent available from Sd fine chemicals, Mumbai.

To 2-3ml filtrate few drops of Wagner's reagent are added. Reddish blue ppt is formed.

Detection of Flavonoids

To small amount of the residue add lead acetate solution. Yellow colored ppt is formed.

Detection of anthocyanosids

Alkaline reagent test

The presence of anthocyanosids is revealed by a color change as a function of pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if blue, the p H is 4 and 6.

Detection of Phenolics and Tannins

100 mg of the extract was boiled with 1ml of distilled water and filtered. The filtrate was used for the following test,

a. Ferric chloride test: To 2ml of filtrate, 2ml of 1% ferric chloride solution was added in a test tube. Formation of bluish black color indicates the presence of phenolic nucleus.

b. Test for Tannins: To the extract 0.5ml NaOH was added formation of precipitate indicates the presence of tannins.

Detection of Phytosterols and Triterpenoids

0.5 g of the extract was treated with 10ml chloroform and filtered. The filtrate was used to test the presence of Phytosterols and Triterpenoids.

a. Leiberman -Burchart test: To the extract, few drops of acetic acid and con H_2SO_4 were added, deep red ring at the junction of two liquids indicates the presence of triterpenes.

b. Salkowaski test: To the extract solution few drops of con H_2S0_4 were added and shaken and allowed to stand, lower layer turns red indicates the presence of sterols.

Detection of fixed oils and fats

Oil spot test: One drop of the extract was placed on filter paper and the solvent was allowed to evaporate. An oily stain on filter paper indicates the presence of fixed oil.

The qualitative chemical tests were performed on the plant extracts to detect the various Phyto-constituents present as per the standard procedures and findings were recorded. The qualitative chemical tests on the various extracts showed that, for ***Mathuka Neriifolia*** (Moon) H.J. Lam. more number of phytoconstituent were found in ethanol, chloroform and water extract. (Table 1)

Table 1: Phytochemical analysis of *Mathuka Neriifolia* (Moon) H.J. Lam. Extracts.

SI/NO	Test	H	P	C	E.A	E	W
1	**Carbohydrates**	-	-	-	-	-	+
2	**Glycosides**	-	-	-	-	+	+
3	**Saponins**	-	-	+	+	+	-
4	**Alkaloids** *Dragendorff's test* *Wagner's test*	- -	- -	- -	- +	- +	- -
5	**Flavonoids**	-	-	-	-	+	-
6	**Aanthocyanosides**	-	-	-	-	+	-
7	**Phenolic and Tannins**	-	-	-	-	+	+
8	**Phytosterols and Triterpenoids** *Salkowaski test* *Leiberman-Burcharat test*	 + +	 + +	 + -	 + -	 - -	 - -
9	**Fixed oils and fats**	+	+	+	-	+	-

- : Absence; +: Presence.
H=Hexane, **P**=Petroleum ether, **C**=Chloroform, **E.A**=Ethyl acetate, **E**=Ethanol, **W**=Water.

Isolation and characterization of phytoconstituents of *Mathuka Neriifolia* (Moon) H.J. Lam. ethanol extract

The *Mathuka Neriifolia* (Moon) H.J. Lam. ethanol, chloroform and water extracts were having maximum number of phytoconstituents. (Table 2).

Table 2: Fractnation of ethanol extract of *Mathuka Neriifolia* (Moon) H.J. Lam. by column chromatography.

Fraction Number	Solvent ratio for column elution	NO. of spots	Rf value
1-4	100% P.E	-Nil-	-Nil-
4-6	P.E 90% : B 10%	-Nil-	-Nil-
6-15	P.E 80%: B 20%	-Nil-	-Nil-
15-30	P.E 70% : B 30%	Three	0.65,0.7,0.4
30-50	P.E 60% :B 40%	-Nil-	-Nil-
50-70	P.E 50% : B 50 %	-Nil-	-Nil-
70-100	P.E 30% : B 70 %	-Nil-	-Nil-
100-120	100% B	-Nil-	-Nil-
121-130	B 90% :C 10%	Three	0.5,0.6,0.9
131-135	B 80%:C 20%	-Nil-	-Nil-
136 – 145	B 50%:C 50%	-Nil-	-Nil-
146 – 166	B 20%:C 80%	-Nil-	-Nil-
167 – 180	B 10%: C 90%	-Nil-	-Nil-
181 – 190	100% C	-Nil-	-Nil-
191 – 195	C99% :M1%	-Nil-	-Nil-
196 – 200	C98%:M2%	-Nil-	-Nil-
201 – 210	C97%:M3%	Three	0.8,0.6,0.4
211 – 230	C96%:M4%	Three	0.8,0.6,0.4
231 – 259	C95.5%:M4.5%	Three	0.8,0.6,0.4
260 – 300	C95%:M5%	One	0.6
301 – 344	C93%:M7%	One	0.7

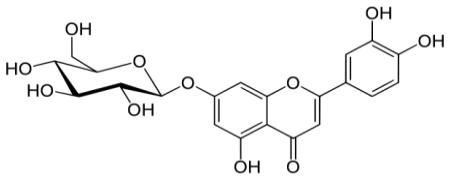
P.E.: Petroleum ether, B: Benzene, C:Chloroform, M:Metanol.

During the column elution process, the fractions 260 - 300 and 301 - 344 has a single banding pattern which was confirmed by TLC study. So the fractions are combined and kept for evaporation to dry at room temperature. After drying the dried residue was scrapped off once again checked for its purity. The remaining fractions were not worked out because of lower yield as well as impure. The compound with Rf value 0.7 was subjected for Phyochemical screening, Physical properties and spectral analysis, i.e., FTIR, LC-MS, C13 NMR and HNMR for structural elucidation. (Table 3) **(Fig.:1)**

Physical properties and other details of isolated compound

- **Color :** Dull ochre
- **M.P:** 256^{0}c
- **TLC single spot**
- **Rf value in BAW by TLC :** 0.69
- **λ max :** 348nm**(Fig.:5)**

- **Bright yellow coloration with ammonia.**
- Shows the presence of **Luteolin 7 - glucoside (Fig.:4)**



Luteolin 7 - glucoside (Fig.:4)

Table 3: Phyochemical screening of component from the ethanolic extracts and fractions of *Mathuka Neriifolia* (Moon) H.J. Lam.

SI/NO	Tests	Component
1	Carbohydrates	-
2	Glycosides	-
3	Saponins	-
4	Alkaloids	-
5	Flavonoids	+
6	Anthocyanosides	-
7	Phenolic and Tannins	+
8	Phytosterols and Triterpenoids	-
9	Fixed oils and fats	-

Interpretation and observation

The compound in its IR spectra exhibited absorption bands at 1000 - 750 cm^{-1} for aromaticity,1500 - 1250 cm^{-1} for alkyl, 1662 cm^{-1}a broadband for ketone and bands at 3406 cm^{-1} and 3315 cm^{-1} for phenolic OH group.**(Fig.:6)**

The LC-MS data shows a peak at retention time 8.1 and molecular weight of 609 in negative mode.(**Fig.:7)**

In its ^{1}H-NMR spectra shows, bands between δ 6 shows aromaticity, δ 7 shows the presence of phenolic OH group, δ 3 shows presence of -CH2-, - CH and an instance peak at δ 5 shows the presence of OH group. (**Fig.:8)**

^{13}C-NMR spectrum exhibits presence of 25 carbon atoms, a signal in the range δ 116 - 179 shows the presence of aromaticity with 12 carbon atoms. So two aromatic rings may be present. An instance band at δ 49 shows the presence of the alkyl group.(**Fig.:9)**

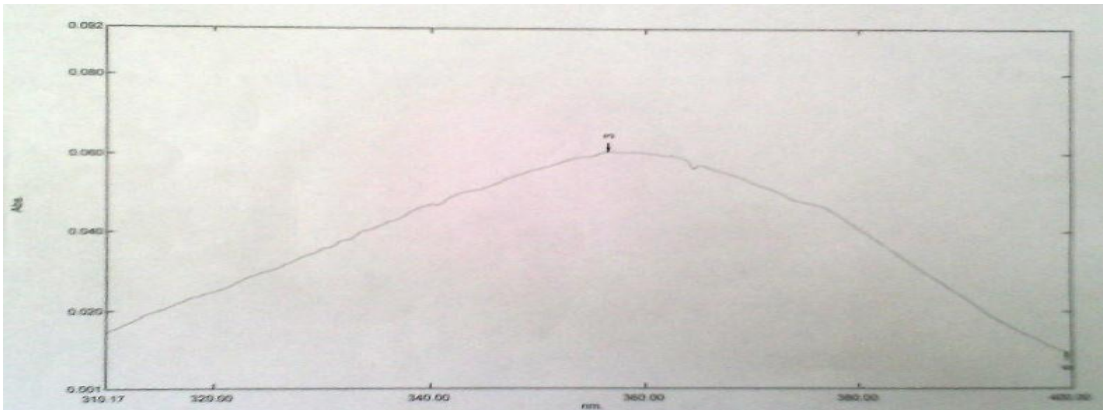


Fig.:5: UV-VISIBLE SPECTRUM OF COMPONENT.

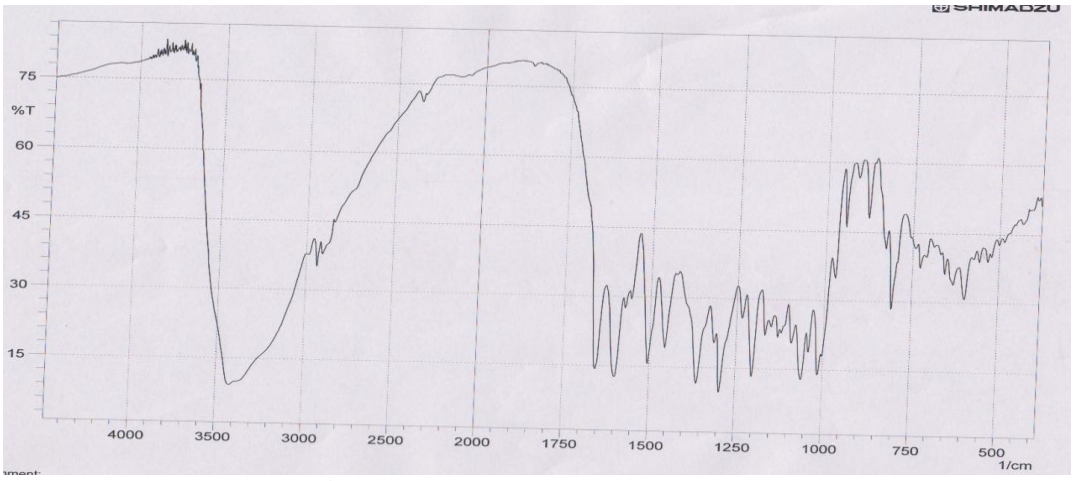


Fig.:6 FTIR DATA OF COMPONENT.

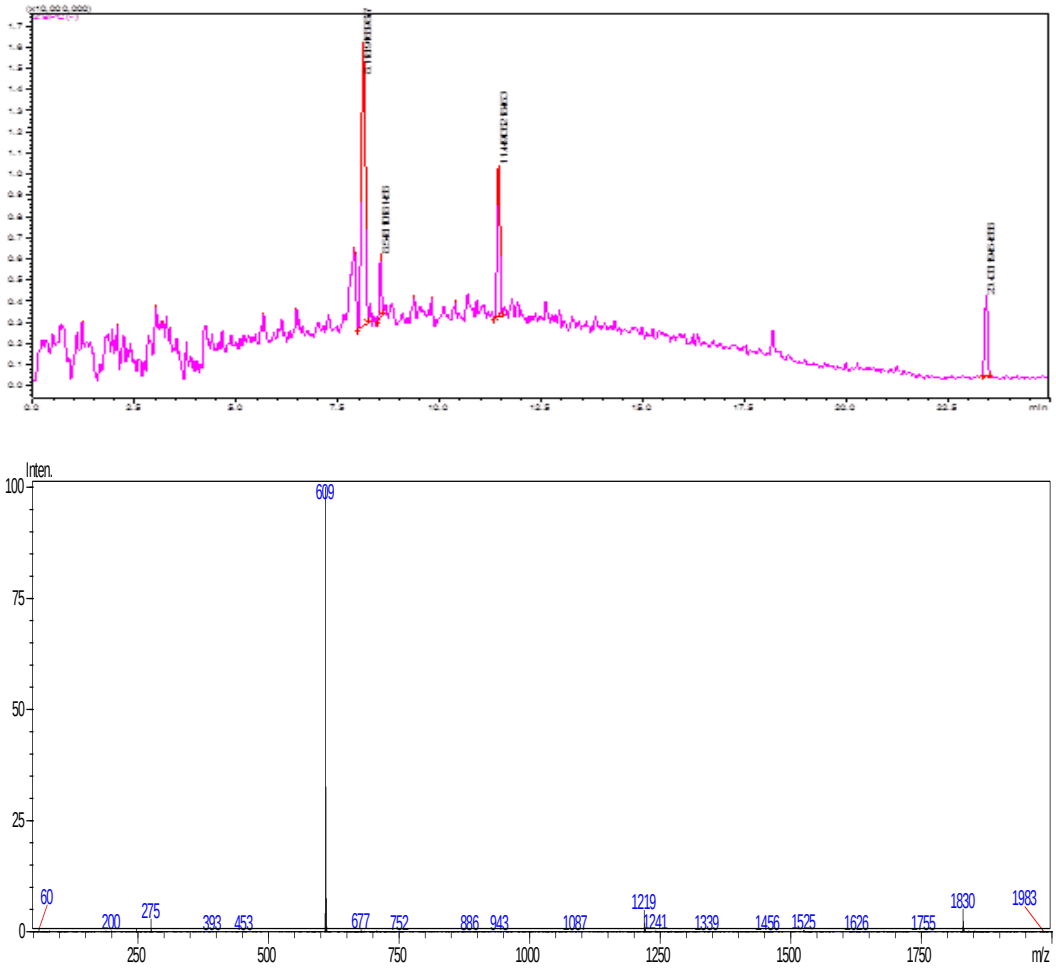


Fig.:7 LC-MS DATA OF COMPONENT.

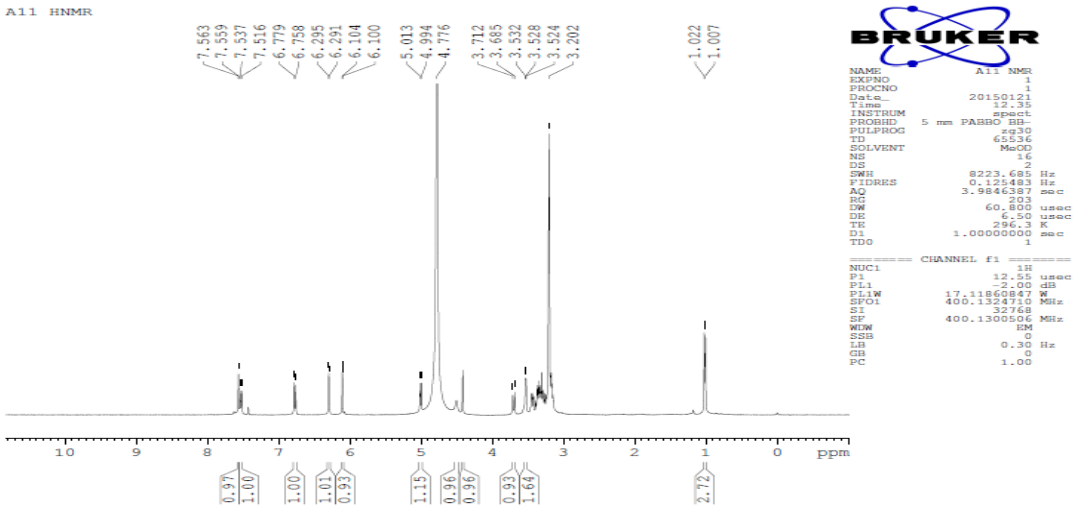


Fig.:8 ^{1}H-NMR DATA OF COMPONENT.

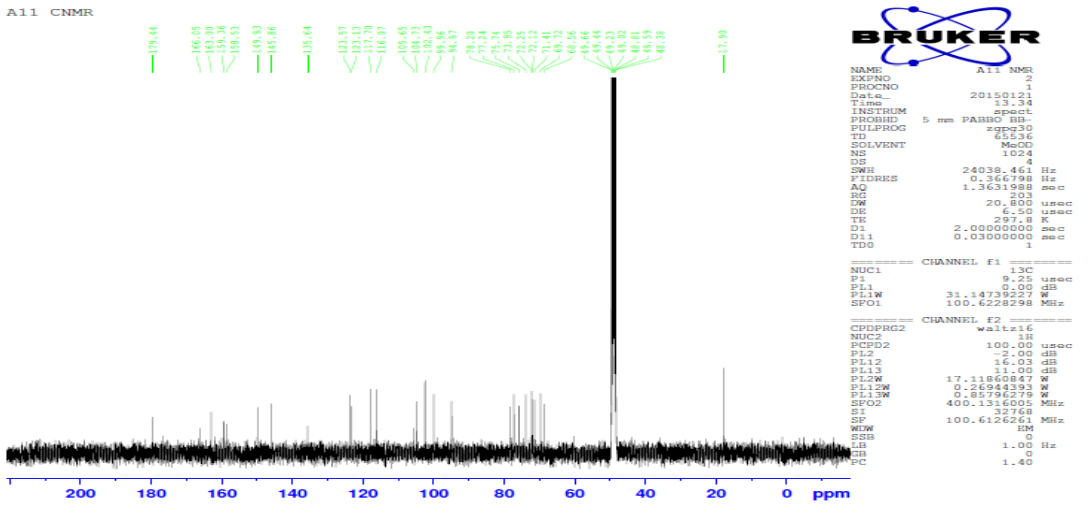


Fig.:9 ^{13}C-NMR DATA OF COMPONENT

RESULTS AND DISCUSSION

Mathuka Neriifolia (Moon) H.J. Lam. Flowers are used in the treatment of kidney complaints. Fruits are recommended in cases of rheumatism, biliousness, asthma and worm trouble. Oil from the seeds are used to treat rheumatism and for improved growth of hair. From the literature, it was found that no other study regarding the antioxidant activity of the plant *Madhuca neriifolia* (Moon) H.J. Lam, has been conducted and as the plant was used to treat rheumatism and other ailments which is a caused due to free radical activity we decide to go for antioxidant study.

The ethanolic extract of *Mathuka Neriifolia* (Moon) H.J. Lam.showed the presence of more phytoconstituents, Alkaloids, Flavonoids, Aanthocyanosides, Phenolic and Tannins and Fixed oils and fats.So further study was conducted with the extract.

Column chromatogtaphy by gradient elution technique was performed and the fractions 260 - 300 and 301 - 344 has a single banding pattern which was confirmed by TLC study. So the fractions are combined and kept for evaporation to dry at room temperature. After drying the dried residue was scrapped off once again checked for its purity. The compound with Rf value 0.7 was subjected for Phyochemical screening, Physical properties and spectral analysis, i.e., FTIR, LC-MS, C13 NMR and HNMR for structural elucidation.

Based on the study the column isolate was confirmed as Luteolin 7 - glucoside

CONCLUSION

Despite the recent interest in molecular modeling, combinatorial chemistry and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products, particularly medicinal plants, remains an important source of new drugs, new drug leads and new chemical entities. It is evident that, natural products have played a vital role in drug discovery, by contributing to a wide variety of phytochemicals for the treatment of cancer, cardiovascular diseases, infections related with viral and microbial origin and other health disorders.

After collection and authentication of plant material, fresh plant of ***Mathuka Neriifolia*** (Moon) H.J. Lam. was shadow dried and powdered. Powdered material was passed through sieve No.60. Then extracted using hexane, petroleum ether, chloroform, ethyl acetate, ethanol by the Soxhlet extraction method. The extracts were concentrated using a rotary vacuum evaporator.

The qualitative chemical tests were performed on the plant extracts to detect the various phyto-constituents present in them as per the standard procedure and findings were recorded. From the qualitative chemical tests, it was found that the chloroform, ethanol and water extracts of ***Mathuka Neriifolia*** (Moon) H.J. Lam. Was having the maximum number of constituents.

So, the ethanol extract was subjected to column chromatography, by means of gradient elution technique. The fractions 260 - 300 and 301 - 344, gave two compounds. Later by spectral analysis they were found that the component one was Luteolin 7 - glucoside**.**

Based on the literature Luteolin 7 - glucoside is a well-known antioxidant present in plants. So as a conclusion further studies with the extracts of *Mathuka Neriifolia* (Moon) H.J. Lam. Will be performed. Also we can conclude that the classical uses confirms the scientific study.

REFERANCES

1. A. J. Stewart, S. Bozonnet, W. Mullen, G. I. Jenkins, M. E. Lean, and A. Crozier, "Occurrence of flavonols in tomatoes and tomato-based products," Journal of Agricultural and Food Chemistry, 2000; 48(7): 2663–2669.

2. A. K. Pandey, "Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weedParihenium histerophorus: an in vitro study," National Academy Science Letters, 2007; 30(11-12): 383–386.
3. Amić, D.; Davidović-Amić, D.; Bešlo, D.; Trinajstić, N. Structure-radical scavenging activity relationships of flavonoids. Croat. Chem. Acta, 2003; 76: 55–61.
4. Annil Mahajan, Vishal R Tandon J. 2004 ;Antioxidants and rheumatoid arthritis. Indian Rheumatol Assoc, 12: 139–142.
5. Bohm, B. Introduction of Flavonoids; Harwood Academic Publishers: Singapore, 1998.
6. Buer, C.S.; Imin, N.; Djordjevic, M.A. Flavonoids: New roles for old molecules. J. Integr. Plant Biol, 2010; 52: 98–111.
7. C. A. Rice-Evans, N. J. Miller, P. G. Bolwell, P. M. Broamley, and J. B. Pridham, "The relative antioxidant activities of plant-derived polyphenolic flavonoids," Free Radical Research, 1995; 22(4): 375–383.
8. Cotelle, N.; Bernier, J.L.; Catteau, J.P.; Pommery, J.; Wallet, J.C.; Gaydou, E.M. Antioxidant properties of hydroxy-flavones. Free Radic. Biol. Med, 1996; 20: 35–43.
9. de la Rosa L.A., Alvarez-Parrilla E., Gonzàlez-Aguilar G.A. Fruit and vegetable phytochemicals: chemistry, nutritional value, and stability. 1th Edition. Wiley J. & Sons, Inc., Publication, 2010.
10. E. H. Kelly, R. T. Anthony, and J. B. Dennis, "Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships," Journal of Nutritional Biochemistry, 2002; 13(10): 572–584.
11. E. J. Middleton, "Effect of plant flavonoids on immune and inflammatory cell function," Advances in Experimental Medicine and Biology, 1998; 439: 175–182.
12. Enos Tangke Arung, Britanto Dani Wicaksono, Yohana Ayupriyanti Handoko, Irawan Wijaya Kusuma, Dina Yulia1 and Ferry Sandra., 2009; Anti-Cancer Properties of Diethylether Extract of Wood from Sukun (Artocarpus altilis) in Human Breast Cancer (T47D) Cells. Tropical Journal of Pharmaceutical Research, 8(4): 317-32.
13. F. Du, F. Zhang, F. Chen et al., "Advances in microbial heterologous production of flavonoids," African Journal of Microbiology Research, 2011; 5(18): 2566–2574.
14. Forkmann, G.; Heller, W. Biosynthesis of flavonoids. In Comprehensive Natural Products Chemistry; Elsevier: Amsterdam, The Netherlands, 1999; 713–748.
15. G. Agati, E. Azzarello, S. Pollastri, and M. Tattini, "Flavonoids as antioxidants in plants: location and functional significance," Plant Science, 2012; 196: 67–76.
16. Han X., Shen T. and Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci, 2007; 9: 950-988. doi:10.3390/i8090950.
17. Hetal Amin**,** Rohit Sharma**,** Mahesh Vyas**,** PK Prajapati**,** Kartar Dhiman**,**2014;Validation of the ayurvedic therapeutic claims through contemporary studies. International journal of green pharmacy, 8(4): 193.
18. Irena Matlawska and Maria Sikorska. 2005; Flavonoids from Abutilon Theophrasti flowers.Acta poloniae Pharmaceutical and drug research, 62(2): 135-39.
19. J B Harborne. 1998;Phytochemical methods, A guide to modern techniques of plant analysis. Third edition. 40-44, 73-79.
20. K. Reinli and G. Block, "Phytoestrogen content of foods: a compendium of literature values," Nutrition and Cancer, 1996; 26(2): 123–148.
21. Khandelwal KR. 2006; Practical Pharmacognosy Techniques And Experiments, 15th ED, Nirali Prakashan, India.
22. Kokate CC, Purohit AP and Gokhale SB. 2001; Text Book of Pharmacognosy, 7th ED, Nirali Prakashan, India.
23. Krishnaiah D, Sarbatly R, Nithyanandam RR., 2011; A review of the antioxidant potential of medicinal plant species. Food Bioprod Process, 89: 217–233.
24. Lorenzo V., Greco and Marco N. Bruno. 2008;Food science and technology:new research. Nova science publishers,. New York, 24-28.
25. M. F. Mahomoodally, A. Gurib-Fakim, and A. H. Subratty, "Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius," Pharmaceutical Biology, 2005; 43(3): 237–242.
26. M. G. L. Hertog, P. C. H. Hollman, and M. B. Katan, "Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands," Journal of Agricultural and Food Chemistry, 1992; 40(12): 2379–2383.
27. M. L. Lázaro, "Distribution and biological activities of the flavonoid luteolin," Mini-Reviews in Medicinal Chemistry, 2009; 9(1): 31–59.
28. M. Leopoldini, N. Russo, S. Chiodo, and M. Toscano, "Iron chelation by the powerful antioxidant flavonoid quercetin," Journal of Agricultural and Food Chemistry, 2006; 54(17): 6343–6351.
29. M. Lopez, F. Martinez, C. Del Valle, C. Orte, and M. Miro, "Analysis of phenolic constituents of biological interest in red wines by highperformance liquid chromatography," Journal of Chromatography A, 2001; 922, no. 1-2: 359–363.
30. Manach C., Scalbert A., Morand C., Rémésy C., and Jime´nez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr, 2004; 79(5): 727-47.
31. Maria Kratchanova, Petko Denev, Milan Ciz, Antonin Lojek and Atanas Mihailov,2010 ;Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds.

Comparison of two extraction systems.ACTA Biochimica polonica, 57, No. 2/ 229–234.
32. Middeltone, H. 1956 ;Systematic Qualitative Analysis. Edward Arnnold Publishers Ltd., London.
33. Murphy Karen J, Chronopoulos Andriana K, Singh I, el al. Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. American Journal of Clinical Nutrition, 2003; 77(6): 1466-73.
34. N. C. Cook and S. Samman, "Review: flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources," Journal of Nutritional Biochemistry, 1996; 7(2): 66–76.
35. Peach T, Trancey MV. Modern Methods in Plant Analysis. Springer Verlog, Berlin, 1955.
36. Prasanna Kumar C N, Shivaprasad D, Somashekar R K, Nagaraja B C., 2013; Reproductive Phenology and Pollination Biology of Madhuca neriifolia in wet evergreen forest of Western Ghats, South India. International Journal of Advanced Research, 1(9): 296-306.
37. R. A. Dixon, P. M. Dey, and C. J. Lamb, "Phytoalexins: enzymology and molecular biology," Advances in Enzymology and Related Areas of Molecular Biology, 1983; 55: 1–136.
38. R. L. Rousseff, S. F. Martin, and C. O. Youtsey, "Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus," Journal of Agricultural and Food Chemistry, 1987; 35(6): 1027–1030.
39. R.B.Kshatriyaa, Dr.G.M.Nazeruddin, bioactive bioactive bioactive flavonoids flavonoids flavonoids of therapeutic importance, 7 th International Symposium on Feedstock Recycling of Polymeric Materials (7th ISFR 2013) New Delhi, India, 23-26 October 2013.
40. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med, 1996; 20: 933–956.
41. Rosenthaler L. 2011; Chemical investigations of plants. G. Bell and Sons, London, 1930.
42. S. Kreft, M. Knapp, and I. Kreft, "Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis," Journal of Agricultural and Food Chemistry, 1999; 47(11): 4649–4652.
43. S. Kumar and A. K. Pandey, "Phenolic content, reducing power and membrane protective activities ofSolanum xanthocarpum root extracts," Vegetos, 2013; 26: 301–307.
44. S. Kumar, A. Gupta, and A. K. Pandey, "Calotropis procera root extract has capability to combat free radical mediated damage," ISRN Pharmacology, vol. 2013, Article ID 691372, 8 pages, 2013.
45. S. Kumar, A. Mishra, and A. K. Pandey, "Antioxidant mediated protective effect of Parthenium hysterophorus against oxidative damage using in vitro models," BMC Complementary and Alternative Medicine, 2013; 13, article 120.
46. Scholars Research Library. 2012; J. Nat. Prod. Plant Resour, 2(4): 512-516.
47. Seyoum, A.; Asres, K.; El-Fiky, F.K. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry, 2006; 67: 2058– 2070.
48. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients, 2010; 2: 1231-1246. doi:10.3390/nu2121231.
49. Y. Hara, S. J. Luo, R. L. Wickremasinghe, and T. Yamanishi, "Special issue on tea," Food Reviews International, 1995; 11: 371–542.
50. Y. Miyake, K. Shimoi, S. Kumazawa, K. Yamamoto, N. Kinae, and T. Osawa, "Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats," Journal of Agricultural and Food Chemistry, 2000; 48(8): 3217–3224.