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Research Article

HPTLC Estimation of Potential Antidiabetic Agent "Corosolic Acid" in Few Notable Members of Order Myrtales

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ABSTRACT
Diabetes is one of the major lifestyle disorders faced by a large fraction of the population today. Type II Diabetes is developed as a result of metabolic disbalance due to the altered functioning of the endocrine system and changes in the metabolism of carbohydrates, proteins, and lipids. Patients with these complications may develop alterations in the functions of different body organs or prolonged illness. Till date, there is immense progress in the treatment of diabetes by oral hypoglycaemic agents, but still, there is a need to search for newer drugs. One reason is that existing synthetic drugs have several limitations and side effects. Therefore, in recent years apart from the allopathic mode of treatment, people are turning towards complementary and alternative medicines (CAM) to treat diabetes. Ethnobotanical information suggests that approximately 800 plants are used in traditional remedies for the treatment of diabetes. However, they are yet to be standardized and commercially formulated. There are many active chemical compounds extracted from the plants for the treatment of diabetes. Corosolic acid is one of the best active ingredients exhibiting potential antidiabetic activity. Earlier extraction of corosolic acid was limited to very few plants, in recent years, due to the availability of advanced analytical methodology lot of plants are being screened for the detection of corosolic acid. Majority of plants screened, are not native to India or its availability is limited. we need to screen more plants to encounter an exponentially increasing pharmaceutical market of corosolic acid, focusing on this aim, we have considered leaves of 16 easily available plant members from the order Myrtales for the screening of corosolic acid. The results reveal that out of 16 Myrtale members the maximum amount of corosolic acid was obtained in *Callistemon lanceolatus* (0.367161%), while the least amount was observed in *Pimenta dioica* (0.018025%), in 4 other members (*Gustavia augusta, Cauropita guianensis, Lawsonia inermis, Ammania baccifera)* corosolic acid was not detected. as we can see from the results there are many myrtle members which contain a greater amount of corosolic acid, our study will influence the screening of more plants for the effective extraction of corosolic acid.

Keywords: Diabetes, Corosolic acid, Myrtales, HPTLC, CAM

INTRODUCTION

Diabetes is a rising health hazard around the globe today, the contemporary lifestyle, unhealthy work environment and stress are the main contributing reasons for the rise in the prevalence of the disease. It is an array of metabolic illnesses characterized by hyperglycaemia, as a consequence of abnormalities in insulin production, insulin action, or a combination of both the factors. It is now well recognized that people who have diabetes are at elevated risk for a variety of major health issues, including cardiovascular disease, early mortality, blindness, renal failure, amputations, fractures, frailty, depression, and cognitive decline.

Till date, there is immense progress in the treatment of diabetes by oral hypoglycaemic agents, but still, there is a need to search for newer drugs. One reason is that existing synthetic drugs have several limitations and side effects. Therefore, apart from the allopathic mode of treatment, people are turning towards complementary and alternative medicines (CAM) to treat diabetes in recent years. Complementary and alternative medicine (CAM) is a growing sensation among the people with chronic illnesses, like hypertension, cardiovascular diseases, depression, including diabetes mellitus etc.[1,2] After WHO recommendations, explained for the effective management of type II diabetes during the year 1980 (IDF 2003), the search for safer and most effective molecules for the treatment of diabetes has continued to be an important area of active research around the world. Ethnobotanical information suggests that approximately 800 plants are used in traditional remedies for the treatment of diabetes.[3] However, they are yet to be standardized and commercially formulated.

In traditional medicine, many plants are used to control blood sugar levels in diabetic patients, such as *Momordica charantia*[4], *Gymnema Sylvestre*[5], Syzygium *cumini*[6], *Lagerstroemia speciosa*[7], *Pterocarpus marsupium*[8] etc. Plant-derived drugs are now gaining attention of researchers throughout the world, however there may be thousands of molecules present in a single plant extract. Therefore, it is tough to identify and standardized the active components in each plant extracts. The lack of standardisation leads to the non-acceptance of the plant derived drugs. Despite the challenges posed by regulatory

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bodies, considerable work is being done to seek new plant-based drugs to treat diabetes. There are different group of plants associated with specific genus, order or families, which are associated with same or slightly different chemical compositions, these group of plants can be utilized for the treatment of different health aliments.

The order Myrtales are a group of dicotyledonous plants, spread across tropical and temperate regions of the world. Members are woody or herbaceous, majority of them are terrestrial or rarely aquatic. Several studies have been conducted to identify the deep lineage divergence in the order Myrtales, still the members placed in this order has varied in different classifications with many divergent opinions.[9] During recent study using "relaxed clock methodology'' dates the crown of order myrtles lineage between 89 to 99 million years ago (Bell *et al.,* 2010). During the year 1862, Bentham and Hooker placed order Myrtales in the series calyciflorae of polypetalae. This group shows a transition from perigyny to epigyny condition in terms of ovary position in the flower. Bentham and hooker placed six families in the order Myrtales, they were Rhizophoraceae, Combretaceae, Myrtaceae, Melastomataceae, Lythraceae and Onagraceae.[10] Engler and Prantle during the year 1887 added 3 more families Punicaceae, Lecythidaceae and Alangiaceae.[11] During the year 1973, Hutchinson the well-known taxonomist placed 10 families in the order Myrtales, he added three families, Barringtoniaceae, Asteranthaceae which was a group separated from Lecythidaceae and Sonneratiaceae separated from the family Lythraceae. The two families Alangiaceae and Onagraceae were removed by Hutchinson.[12] In recent studies concluded by Thome in the year 2000, the order Myrtales was divided into three suborders Melastomatineae, Myrtineae and Lythrineae. The suborder Melastomatineae includes seven different families (Penaeaceae, Oliniaceae, Rhynchocalycaceae, Alzateaceae, Crypteroniaceae, Melastomataceae and Memecylaceae), suborder Myrtineae includes three different families (Myrtaceae, Onagraceae and Vochysiaceae) and suborder Lythrineae also consists of three families namely Lythraceae, Onagraceae and Combretaceae.[13] There are thousands of members included in order Myrtales, many are considered to be economically important in terms of different products obtained from them 1) High-density timber can be obtained from many *Eucalyptus* species like *Eucalyptus camaldulensis* (Red gum), *Eucalyptus globulus* (blue gum), *Eucalyptus astringens* (brown Mallet) etc. 2) Myrtales members are well known for its valuable fruits which are used around the world example, Pomegranate (*Punica granatum*), Guava (*Psidium guajava*), Grumichama (*Eugenia brasiliensis*), Pitanga (*Eugenia uniflora*), mountain apple (*Syzygium malaccense*) etc. 3) Many plants (*Pimenta dioica, Eucalyptus globulus, Syzygium aromaticum* etc.) yield economically valuable volatile oils. 4) Tannins are majorly extracted from the members of family Myrtaceae and Rhizophoraceae. 5) Majority of Myrtales members are also used as medicinal plants, some of the most important members are *Terminalia arjuna, Terminalia chebula, Woodford fruticose, Syzygium aromaticum, Sonneratia apetala, Lagerstroemia speciosa* etc.[14]

The order Myrtales represents fairly homogenous complexes in terms of their chemical composition. All families of order Myrtales confirm the presence of tannins as a major phytochemical. During the year 1984 Hegnauer concludes that, the gallic acid, ellagic acid and tannins, which are derived from flavon-3-oles and flavon-3-4 dioles are one of the most important characteristic features of an order Myrtales.[15] If we consider flavonoids in order Myrtales, they are mainly common flavonols and their O-methyl derivatives eg., Cyanidin, quercetin, kaempferol, anthocyanin etc.[16] The presence of alkaloids are scattered in the order Myrtales they are majorly present in the member of Combretaceae, Lythraceae, Melastomataceae and Myrtaceae. Triterpenes are widely distributed in the majority of members in the order Myrtales α-amyrin, β-amyrin, neolupenol, gammacer-16-en-3β-ol, taraxerol, ursolic acid and a few members contain one of the most important therapeutic agents used in diabetes corosolic acid.[17]

Corosolic acid is one of the best active ingredients exhibiting potential antidiabetic activity.[18] The compound also exhibits antihyperlipidemic, anticancer, antioxidant, antifungal, antiviral, antineoplastic and osteoblastic activities.[19,20] Due to its most promising hypoglycaemic effect, both extract from plants and purified corosolic acid have been extensively studied for the treatment of diabetes. Earlier extraction of corosolic acid was limited to very few plants, in recent years, due to the availability of advanced analytical methodology lot of plants are being screened for the detection of corosolic acid some of them are *Phlomis umbrosa*,[21] *Potentilla chinensis*,[22] *Glechoma longituba*,[23] *Weigela subsessilis*,[24] *Eriobotrya japonica*[25-27] *Ugni molinae*,[28] *Symplocos* paniculate[29] etc. if you look at the entire list of plants used for the extraction of corosolic acid, majority of them are not native to India or its availability is limited. Till now a lot of studies have supported the positive use of corosolic acid in the treatment of diabetes Still, there is a need for more research to prove the effective and appropriate use of corosolic acid. It has the potential to emerge as a leading phytochemical which can supplement the treatment of diabetes. Considering its future prospect, we need to screen more plants to encounter an exponentially increasing pharmaceutical market of corosolic acid, focusing on this aim, we have considered many easily available plant members from the order Myrtales for the screening of corosolic acid.

List of plant species from order Myrtales used for corosolic acid analysis, 1. *Eucalyptus globulus, 2. Psidium guajava, 3. Careya arborea, 4. Sonneratia apetala, 5. Melaleuca Leucadendron, 6. Pimenta dioica, 7. Syzygium jambos 8. Syzygium cumini, 9. Callistemon lanceolatus, 10. Barringtonia asiatica, 11. Barringtonia acutangular, 12. Gustavia augusta, 13. Cauropita guianensis, 14. Lawsonia inermis 15. Woodfordia fruticose, 16. Ammania baccifera*.

MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected from different regions of Mumbai and suburbs. The leaves of all 16 members of order Myrtales (*Eucalyptus globulus, Psidium guajava, Careya arborea, Sonneratia apetala, Pimenta dioica, Melaleuca Leucadendron, Syzygium cumini, Syzygium jambos, Callistemon lanceolatus, Barringtonia acutangular, Barringtonia asiatica, Gustavia augusta, Cauropita guianensis, Lawsonia inermis Woodfordia fruticose, Ammania baccifera)* were collected and properly cleaned to eliminate dirt or dust particles; all materials were allowed to air dry in the shade before, it was pulverized to a coarse powder, after crushing, it was sieved with (180 µm) sieve, then the powdered samples were kept in airtight containers for further use.

Preparation of solvent extract

To prepare each plant leaf extract, coarse powder (3 g) of each plant was refluxed in a Soxhlet apparatus twice with 200 mL of methanol for 6 hours. All of the extracts were collected and vacuum dried at low pressure. the dried extracts were weighed, and then the extract was reconstituted with ten times the solvent volume (1:10 w/v extract to solvent). The resultant extracts were used for the HPTLC method development and Corosolic acid analysis.

Preparation of Stock Solutions

Preparation of stock (A) solution of corosolic acid (1000 µg/mL)

The stock solution of corosolic acid was made using HPLC grade methanol, 10 mg of the standard was weighed and transferred to 10 ml of standard volumetric flask. The standard was first dissolved in 5 ml of Methanol, then sonication for 10 minutes and the total volume was raised to 10 ml using methanol.

Sample application and development of chromatogram

The samples were spotted with a Camag microliter syringe (100 µl) on a pre-coated silica gel aluminum plate 60 F254 (100 mm x 200 mm) with 250 µm thickness (E. Merck, Darm Stadt, Germany) using a Camag Linomat V (Camag, Switzerland) applicator. Linear ascending development was carried out in 100mm x 200mm twin trough glass chamber (Camag, Switzerland), mobile phase consists of Toluene: Ethyl acetate: Glacial acetic acid (11:5:0.5 v/v) saturated for 20 minutes. The length of the chromatogram run was 8 cm. the plate was air-dried for 15 minutes and sprayed with 10% ethanolic sulphuric acid. The bands were developed after heating it in a hot air oven at 110 °C for 8 min. The TLC plates were scanned using a Camag TLC scanner at 540 nm, controlled by winCATS software Version 4.03.

Method validation

In recent years, HPTLC has been employed as a cutting-edge approach for isolating distinct components from a mixture. High-performance thin-layer chromatography is a refined version of thin-layer chromatography that allows more precise analysis of chemical compounds (CAMAG, 2010). This is one of the advanced techniques used to standardize active chemical compounds from many medicinal plants across the globe.[30] A comprehensive validation approach used to assess the appropriateness of the newly created technique. We have verified HPTLC technique for its specificity, sensitivity, accuracy, precision, repeatability, and robustness (ICH, 2005).

Estimation of Corosolic acid

To estimate the amount of Corosolic acid from different members of order Myrtales, 5 µl of different plant extracts with Corosolic acid was spotted on the TLC plates (band length 8mm). The chromatographic plate was developed using a solvent system (Toluene: Ethyl Acetate: Glacial Acetic acid in the ratio of 11: 9: 0.5 v/v) till it reaches 8 cm. chromatographic plate was air-dried and sprayed with 10% ethanolic sulphuric acid reagent. After the development of chromatographic plate, it was kept in a hot air oven at 110 °C (8 min), for the development of visible bands. The plate was scanned at 540 nm wavelength of light using a CAMAG TLC scanner. The quantitative evaluation was calculated using their respective HPTLC chromatogram percentage area.

RESULT

Considering our discussion regarding the importance of corosolic acid in the treatment of diabetes, as we know the more efficient studies will lead to an exponential increase in the demand for corosolic acid, in our studies we have screened a few easily available members of order Myrtales for corosolic acid content, so that we can have multiple sources of this phenomenal Phyto molecule. All the parameters involved in method validation was evaluated and most of them were experimentally significant, the details are represented in table 1.

The quantity of Corosolic acid was calculated using their respective calibration curves compared with peak areas. The results (Table 2) reveal that out of 16 Myrtale members the maximum amount of corosolic acid was obtained in *Callistemon lanceolatus* (0.367161%), *Psidium guajava* (0.319115%), *Melaleuca Leucadendron* (0.187087%) and *Woodfordia fruticose* (0.178839 %) respectively, while the least amount was observed in *Pimenta dioica* (0.018025%), in 4 other members (*Gustavia augusta, Cauropita guianensis, Lawsonia inermis, Ammania baccifera)* corosolic acid was not detected.

Table 1: Summary of HPTLC method validation parameters.

Parameter	Corosolic Acid
Linearity(µg/ml)	100-700 (µg/mL)
Correlation coefficient	0.9949
LOD (µg/mL)	9.077 µg/mL
LOQ (µg/mL)	27.506 µg/mL
Precision (RSD)	≤ 2 %
Specificity	Specific
Robustness	Robust
Retention Factor	0.32 ± 0.0042

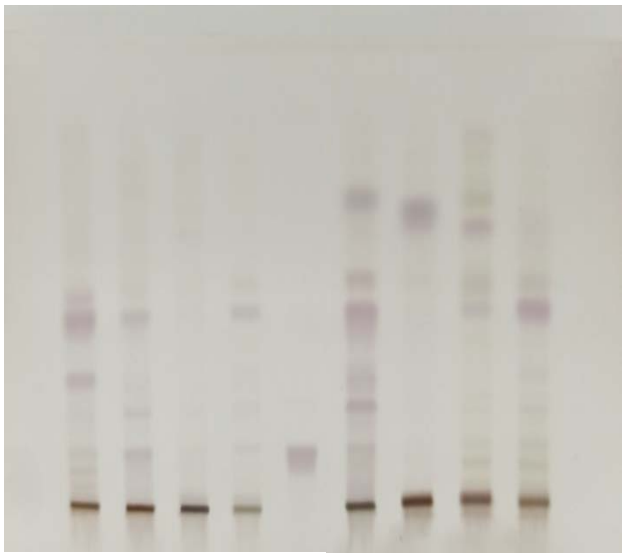


Figure 1: HPTLC Fingerprint at wavelength 540 nm (*B1-E.g; B2 -P.g; B3 -C.a; B4-S.a; B5- Corosolic acid; B6-P.d; B7-M.l; B8-S.c; B9- S.j)*

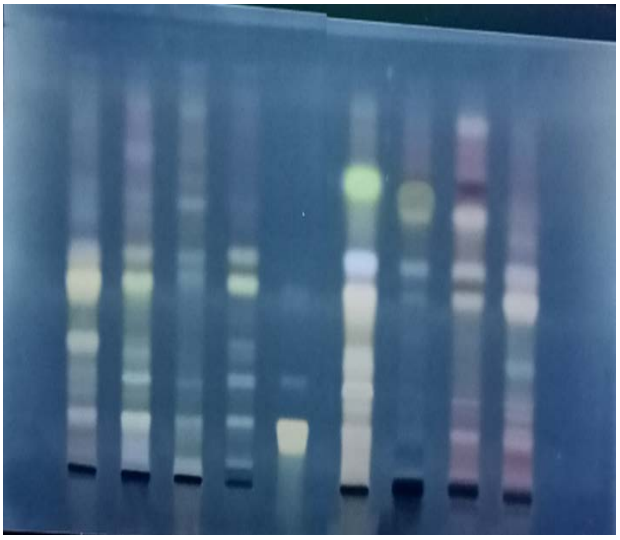


Figure 2: HPTLC Fingerprint at wavelength 366 nm (*B1-E.g; B2 -P.g; B3 -C.a; B4-S.a; B5- Corosolic acid; B6-P.d; B7-M.l; B8-S.c; B9- S.j*

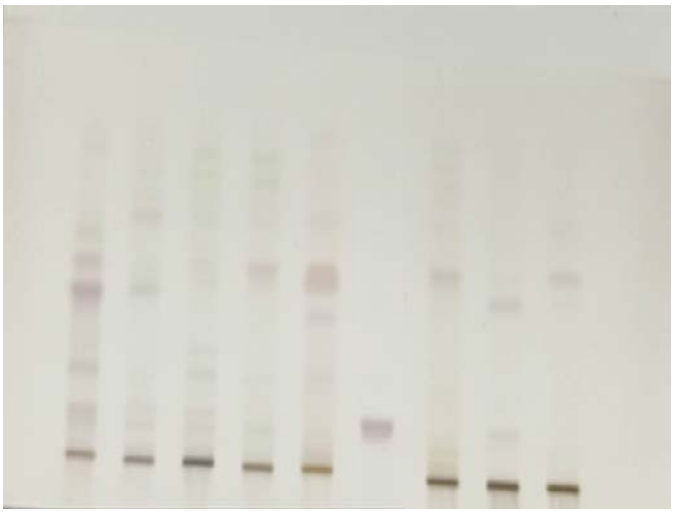


Figure 3; HPTLC Fingerprint at wavelength 540 nm (B1-C.l; B2 – B.a; B3 -B.asi; B4-G. a; B5- C.g; B6- Corosolic acid; B7-L.i; B8-W.f ; B9-A.b)

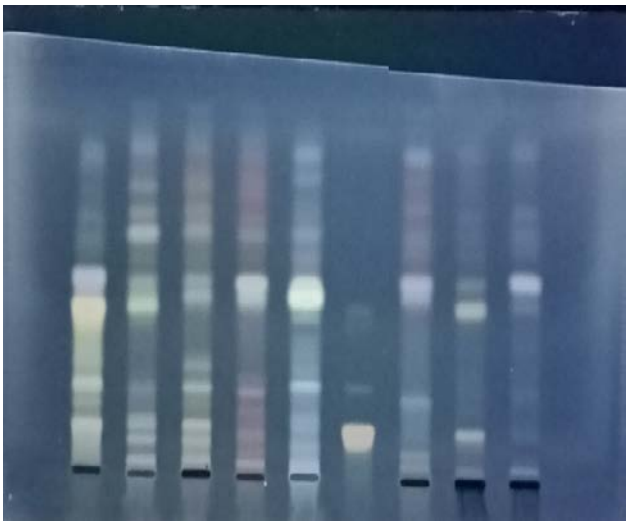
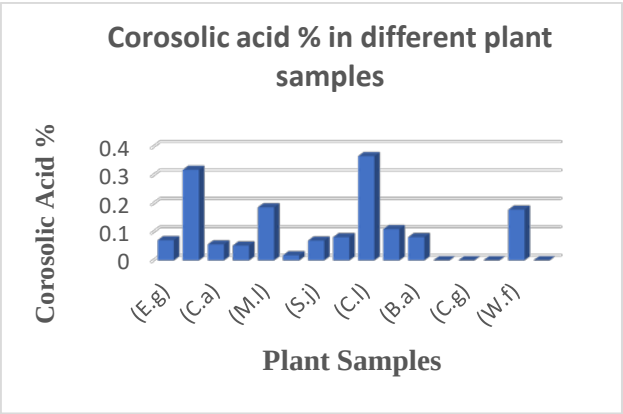


Figure 4: HPTLC Fingerprint at wavelength 366 nm (B1-C.l; B2 – B.a; B3 -B.asi; B4-G.a; B5- C.g; B6- Corosolic acid; B7-L.i; B8-W.f ; B9-A.b)



Graph 1: Quantification of Corosolic acid from different notable plants, belonging to order Myrtales

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Table 2: Quantification of Corosolic acid from different notable plants, belonging to order Myrtales.

Sr No	Plant Samples	Family	Corosolic Acid %
1	*Ammania baccifera (A.b)*	Lythraceae	Not detected
2	*Barringtonia acutangular (B.a)*	Lecythidaceae	0.08191
3	*Barringtonia asiatica (B.asi)*	Lecythidaceae	0.110095
4	*Callistemon lanceolatus (C.l)*	Myrtaceae	0.367161
5	*Careya arborea (C.a)*	Lecythidaceae	0.056418
6	*Cauropita guianensis (C.g)*	Lecythidaceae	Not detected
7	*Eucalyptus globulus (E.g)*	Myrtaceae	0.071196
8	*Gustavia augusta (G.a)*	Lecythidaceae	Not detected
9	*Lawsonia inermis (L.i)*	Lythraceae	Not detected
10	*Melaleuca Leucadendron (M.l)*	Lythraceae	0.187087
11	*Pimenta dioica (P.d)*	Myrtaceae	0.018025
12	*Psidium guajava (P.g)*	Myrtaceae	0.319115
13	*Sonneratia apetala (S.a)*	Myrtaceae	0.052284
14	*Syzygium cumini (S.c)*	Myrtaceae	0.081632
15	*Syzygium jambos (S.j)*	Myrtaceae	0.069757
16	*Woodfordia fruticose (W.f)*	Lythraceae	0.178839

DISCUSSION

Diabetes is an epidemic lifestyle disorder affecting millions of people around the globe, frequent use of antidiabetic chemical agents and insulin is very expensive and it may cause long time side-effects to the human body. In recent years corosolic acid is one of the extensively studied phytochemicals, which can be used for the treatment of diabetes. Corosolic acid follows various aspects of glucose metabolism including enhanced cellular uptake of glucose with the help of stimulating glucose transporters, impaired hydrolysis of sucrose and starches and decreased gluconeogenesis. The effect of corosolic acid is extensively studied in vitro, animal model systems and human subjects, no significant adverse effects have been reported in animal studies or controlled human clinical trials. Considering all these factors, there is a possibility that the demand of corosolic acid is going to exponentially increase in the future, to full fill this demand we need to find may more sources of corosolic acid in the members of plant kingdom. In our studies we have screened a few members of order Myrtales, we have observed that *Callistemon lanceolatus (*0.367161%), *Psidium guajava (*0.319115%), *Melaleuca Leucadendron (*0.187087%) and *Woodfordia fruticose (*0.178839 %) can also be an excellent source of corosolic acid.

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