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PHYTOCHEMICAL AND GC-MS ANALYSIS IN *GRONA TRIFLORA* (L.) H.OHASHI & K.OHASHI (FABACEAE)

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Abstract

Herbal medicine has grown rapidly worldwide because of its natural origin and fewer reported side effects, leading pharmaceutical industries to face challenges in raw material availability, authentication, standardization, and quality control. Standardization is a crucial step to ensure consistent biological activity, a defined chemical profile, and overall quality through organoleptic, physical, chemical, biological, and botanical evaluations. In this study, *Grona triflora* (family Fabaceae), a widely distributed medicinal plant, was selected to evaluate its pharmacognostic, physicochemical, phytochemical, and anti-inflammatory properties. The dried plant powder was analyzed for organoleptic characters such as colour, nature, odour, and taste, along with physicochemical parameters including moisture content, swelling index, foaming index, pH, ash value, and extractive value to assess quality and purity. The plant exhibited low moisture content (9.71%), indicating good stability and low microbial risk, while an ash value of 9.73% suggested minimal inorganic impurities and acceptable purity. Phytochemical screening confirmed the presence of saponins, tannins, terpenoids, alkaloids, flavonoids, and glycosides, compounds known for diverse therapeutic activities. Anti-inflammatory activity evaluated by protein denaturation assay showed higher inhibition by the plant extract compared to diclofenac, indicating strong anti-inflammatory potential. Furthermore, GC–MS analysis identified 70 bioactive compounds, highlighting the medicinal significance of *Grona triflora*, though further pharmacological studies are required to validate the efficacy and mechanisms of individual constituents..

Keywords : Pharmaceutical , Physicochemical Organoleptic

Introduction

Medicinal plants have been a fundamental aspect of human civilization for centuries, serving as natural treatments for a variety of health issues and maintaining a significant role in contemporary medicine. The wisdom surrounding these plants has been transmitted through generations, establishing the basis for traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and various Indigenous healing practices globally. Previous studies have emphasized the importance of medicinal plants as primary sources of healthcare in rural and indigenous communities, where access to modern medicine is limited (Farnsworth & Akerele, 1985). As awareness of natural health alternatives grows, the relevance of medicinal plants in modern healthcare remains strong.

Pharmacognosy has been developed from ancient civilization who used parts of plants and animals for healing, eliminate pain, control suffering and to treat diseases. Pharmacognosy, the scientific study of medicinal drugs derived from natural sources, has long served as the foundation for the discovery and development of plant-based therapeutics (Samuelsson, 2004). It is a science devoted to the study of medicines of natural origin of animal, mineral or vegetable origin. Scientific research carried out on medicinal plants has enabled the isolation of more than 170,000 bioactive molecules from which almost 70% of our current pharmaceutical products originate (Newman, & Cragg, 2004).

The characteristic of a substance or treatment that alleviates inflammation or swelling is known as anti-inflammatory or antiphlogistic. Anti-inflammatory medications, commonly known as anti-inflammatories, constitute nearly fifty percent of pain relievers. Several medicinal plants have demonstrated significant anti-inflammatory properties in previous studies, making them valuable sources for developing safer alternatives to synthetic anti-inflammatory drugs (Pan, Lai, & Ho, 2010). These medications alleviate pain through the reduction of inflammation, unlike

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opioids, which inhibit pain signals to the brain by influencing the central nervous system. A medication or substance that alleviates inflammation, which includes symptoms like swelling, redness, and pain, in the body. Anti-inflammatory medications prevent specific elements within the body that lead to inflammation. They are employed to address a variety of medical issues. Research is being conducted on various anti-inflammatory drugs for their potential use in both the prevention and management of cancer.

Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical technique that combines the features of gas chromatography and mass spectrometry to identify and quantify compounds in complex mixtures. It is widely used in the fields of pharmacognosy, forensic science, environmental analysis, food safety, and drug development. In GC-MS, the sample is first vaporized and separated in the gas chromatograph based on the volatility and interaction of its components with the column's stationary phase. These separated components then enter the mass spectrometer, where they are ionized, fragmented, and detected based on their mass-to-charge ratios. This process allows for precise identification of individual compounds even in minute concentrations. In medicinal plant research, GC-MS is especially valuable for profiling phytochemicals such as essential oils, alkaloids, terpenoids, and fatty acids (Sethi, 2007). It aids in discovering bioactive compounds with therapeutic potential, including those with antimicrobial, antioxidant, or anti-inflammatory properties (Kumar, Yadav& Kumar, 2017). One of the key advantages of GC-MS is its high sensitivity and specificity, which make it suitable for analysing volatile and semi-volatile compounds. However, it has limitations in analysing thermally unstable or non-volatile components, which are better suited for techniques like LC-MS. Despite this, GC-MS remains one of the most reliable and widely used tools for qualitative and quantitative analysis in natural product chemistry.

The plant selected for the investigation is *Grona triflora* belonging to the family fabaceae. *Grona triflora*, commonly referred to as creeping tick trefoil (Joshi, , 2004) or three-flower beggarweed. This plant is indigenous to tropical areas worldwide and has been introduced to subtropical regions, such as the southern United States. *Grona triflora* is prevalent across the tropical and subtropical regions of the world, reaching into continental areas that experience significant temperature variations (Staples & Herbst, 2005). Efforts have been made to delineate the boundaries of its native range; however, these boundaries are somewhat obscured by the spread of naturalized populations. This species is a key component of short (grazed) native and cultivated pastures, where it can constitute up to 50% of the total herbage. The creeping mat it forms offers effective ground cover during the wet season, particularly in areas that are mown or closely trimmed. It sustains a stable ground cover in conjunction with stoloniferous grasses under conditions of continuous heavy grazing or regular mowing, such as in plantation crops and lawns. Additionally, it is regarded as possessing medicinal properties for the treatment of dysentery, rheumatism, fever, jaundice, stomach pain, skin ailments, wounds, and ulcers, particularly in India and China. It thrives in a variety of soil types, including acidic soils with high aluminium content (Pengelly & Maass, 2001). The aim of the present work is a systematic study on the phytochemical and biological activity to validate the medicinal potential of *Grona triflora.*

Materials and Methods

Collection of Plant material

The plant selected for the present study *Grona triflora* was collected from Kollam. The whole plant was used for the project work.

Powder analysis

Fresh plant of grona trifloral was collected in a polythene bag. The collected material was washed under tap water to remove dirt. Then it was shade dried and powdered in a mixed grinder and sieved with a fine mesh sieve. The powder was then used for further study

Organoleptic study

Organoleptic (literally "impression on the organs") refers to the evaluation by means of the organs of sense and includes the

macroscopic appearance of the plant material, its colour, odour, and taste, occasionally the sound of 'snap' of its fracture and the 'feel' of the powder to the touch (Wozniak et al., 1997). The plant powder characteristics like the odour, taste, colour and nature were evaluated.

Physiological Characterization

Different physiochemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.

1.Loss on drying (Indian pharmacopoeia ,1992)
2. Total ash
3. Acid-insoluble ash
4. Water-soluble ash
5. Sulphated ash
6. Alcohol-soluble extractive
7. Water-soluble extractive
8. pH (Iqbal et.al , 2010)
9. Swelling index (WHO, 1992)
10. Foaming index (WHO,1992)

Phytochemical Screening

Preparation and yield of extract (Indian Pharmacopoeia , 1996)

About 15 g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100 ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

Percentage yield (%) = (weight of the crude extract/weight of the sample) X 100

Qualitative Analysis

A total of 15 phytochemical constituents were tested using standard biochemical procedures (Harborne, 1973).

GCMS Analysis

The analysis of the extract was performed using GC-MS (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.51 ml/min. injector and mass transfer line temperature were set at 200°C and 240°C respectively. The oven temperature was set from 70 to 220°C at 10°C/min, held isothermal for three minutes and finally raised to 300°C at 10°C/min. Two microlitres of the sample was injected in a split mode with a scan range of 40 – 1000 m/z. The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Biological activity - Anti-inflammatory property

Protein denaturation assay

The reaction mixture (0.5 ml) consisted of 0.4 ml bovine serum albumin (3% aqueous solution) and varying concentrations of test sample. The samples were incubated at 37ºC for 20 min and 1 ml phosphate buffered saline (pH 6.3) was added to each tube and then heated at 80ºC for 10 min. The absorbance was measured using spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated as follows:

Percentage of inhibition =
[(Abs Control – Abs Sample) / Abs control)] x100

Statistical analysis

Experimental results were analysed statistically using regression analysis.

Results

Powder analysis

Organoleptic study

The plant powder characteristics like colour, odor, taste and texture of *Grona triflora* was determined (Table 1).

Table 1 . Organoleptic characters of *Grona triflora*

Characters	*Grona trifloral*
Colour	Green
Odor	Aromatic/ Green plant smell
Taste	Bitter
Texture	Rough

Physico-chemical characterization

A total of 10 physiochemical parameters were evaluated in *grona triflora* . Ash values indicate

the presence of mineral content and purity. The moisture content in the sample is 9.71%. For herbal medicines, moisture content should be less than 14%, as this helps avoid chemical changes and reduces the chance of microbial growth. pH of the extract in solution is 4.77 which is slightly acidic in nature.). Foaming index of 100 and more indicates the presence of saponins. Saponins act as chemical barrier or shield in the plant defense system to counter pathogens and herbivores.

Table 2 . Physio chemical characters of *Grona triflora*

Tests	***Grona triflora***
Alcohol soluble extract	11.69 %
Water soluble extract	14.57 %
Total ash	9.73 %
Acid insoluble ash	3.99 %
Water soluble ash	0.95 %
Sulphated ash	13.23 %
Loss on drying at 105°C	9.71 %
pH	4.77
Swelling index	3 ml
Foaming index	100

Phytochemical Screening
Yield of Extract

The methanol extract was prepared by Soxhlet extraction. The yield of the methanol extract was 4.1%.

Qualitative Analysis

A total of 11 phytochemicals were qualitatively assessed. Phenols, steroids, carbohydrates, quinones and proteins were absent in plant extract. Saponins, tannins, terpenoids, alkaloids, flavonoids, glycosides were detected in methanol extract of *Grona triflora*.

Anti-inflammatory activity

Protein degradation is considered to be the cause of inflammation and this assay reflects the ability of the extracts to block protein denaturation. Anti-inflammatory analysis presents a comparison between the anti-inflammatory effects of a standard drug and a plant sample across concentrations from 20 to 100 µg/mL, based on percentage inhibition. Both the standard and the sample show a consistent, dose-dependent increase in inhibition (figure 1 and figure 2). While the standard starts at 14.36% inhibition at 20 µg/mL and reaches 93.33% at 100 µg/mL, the sample shows higher inhibition at all concentrations, beginning at 36.72% and peaking at 94.37%. The IC_{50} value of the standard was 57.5 µg/mL, whereas IC_{50} value of the Test Sample was 41.37µg/m. This indicates that the plant extract demonstrates anti-inflammatory activity a little higher than the standard across all tested concentrations. (Table 4 & 5).

Table 3. Phytochemicals tested in *Grona triflora*

Tests	***Grona triflora***
Saponins	+
Tannins	+
Phenols	-
Terpenoids	+
Alkaloids	+
Flavanoids	+
Steroids	-
Glycosides	+
Carbohydrates	-
Quinones	-
Proteins	-

Table 4. Anti- inflammatory activity

Concentration	Percentage of inhibition by standard	Percentage of inhibition by sample
20	14.36	36.72
40	26.67	67.60
60	51.28	72.12
80	77.44	85.18
100	93.33	94.37

Regression analysis was carried out for methanol extract of *Grona triflora* and

and Diclofenac (standard) and the values were recorded (Table 5). The result of R^2(>0.1) showed statistical significance and indicated an adequate goodness of fit.

GCMS Analysis (Gas Chromatography-Mass Spectrometry)

The present study of GC-MS revealed the presence of 70 active compounds. The phytoconstituents identified were mainly sterols, terpenoids and alkanes. One of the major constituent detected was D-Fructose, 3-O -methyl. The second major compound was 9,12 -Octadecadienoic acid (Z,Z) -. The compound with minimum peak was Di(1-methyl-1-silacyclobutyl) amine.

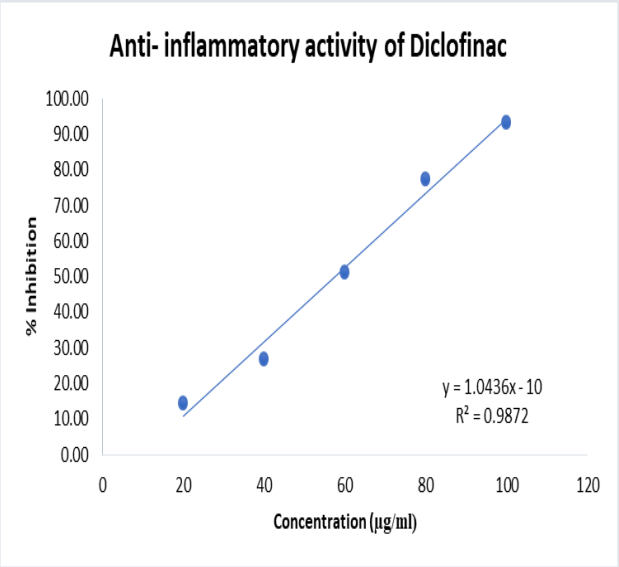

Figure 1. Anti-inflammatory analysis

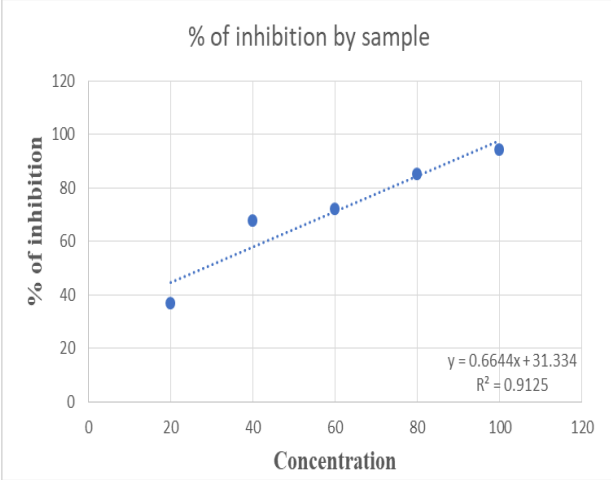

Figure 2. Anti-inflammatory activity of sample

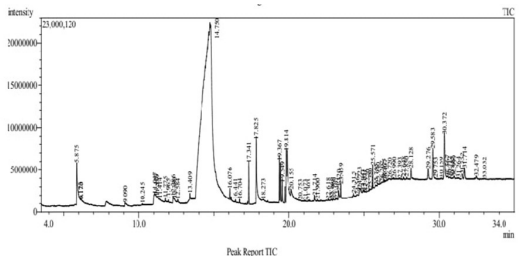

Figure 3. GC-MS Chromatogram

Discussion

Grona triflora is a fast growing, small herbaceous plant belonging to the fabaceae family typically found in tropical and subtropical areas. It commonly thrives in open fields, along roadsides, and in areas that have been disturbed. The plant is distinguished by its three parts leaves, bluish purple flowers, and sprawling growth patterns. Renewed for its traditional medicinal properties, *Grona triflora* is employed to treat wounds, fevers and other digestive ailments. *Grona triflora* is often used in ethnobotanical and phytopharmacological studies (Jain,1981) and evaluation of antimicrobial and cytotoxic activity (Saha, Mandal &Pal, 1995). It is often used as a staple for natural ground covering, due to its durability and quick growth.

Organoleptic evaluation is the process of using our senses—like sight, smell, taste, and touch to check the quality and identity of natural products (Arya & Thakur, 2012), especially in the study of medicinal plants. It is one of the oldest and simplest ways to examine herbal materials. This method helps identify features such as color, smell, texture, and taste, and can also detect if the material is impure or has been mixed with something else. Although it is not as exact as laboratory tests, organoleptic evaluation is still a useful, fast, and affordable way to do an initial quality check of herbal medicines. Organoleptic study was conducted using *grona triflora* powder. It has given green colour, aromatic smell, bitter taste and rough texture (Table 1).

Physicochemical characters of medicinal plants are the physical and chemical features that help check the quality, purity, and identity of herbal materials. These features are important for

making sure plant-based medicines are safe, effective, and consistent. Physicochemical parameters such as moisture content, ash values, and extractive values are essential for the standardization and quality control of herbal drugs (Kokate ,2008) Some common physicochemical tests include measuring moisture content, ash values (like total ash and acid-insoluble ash), extractive values in water or alcohol, and the pH of plant extracts. Moisture content shows how much water is in the plant, which affects storage and the risk of mold. Ash values help find out if the plant has any dirt or unwanted substances. Extractive values help identify how much of the useful compounds can be pulled out using different solvents. Other tests like foaming index, swelling index, checking for foreign matter, and fiber content also help in examining the plant's quality. These tests support standard production, improve shelf life, and make sure herbal products meet safety rules. Studying these characters is a key part of quality control in both traditional and modern herbal medicine.

Physiochemical characters were determined in *Grona triflora* (Table 2). A total of 10 physiochemical parameters were evaluated in *Grona triflora* . Physiochemical characters like ash value, extractive values, loss on drying, foaming index, swelling index, and pH were determined. Loss on drying (LOD) is a method used to find the moisture content in a sample by heating it below its melting point in an oven. This removes water and other substances which can evaporate. Since LOD is not specific, it can also remove other volatile impurities along with moisture. The results of drying are temperature dependent and how long the sample is heated. For herbal medicines, moisture content should be less than 14%, as this helps avoid chemical changes and reduces the chance of microbial growth (Kokate, 2012). Low moisture also stops the growth of microbes like fungi, bacteria, yeast, and mites, which helps improve the safety and shelf life of the product. Moisture content of *Grona triflora* was analysed by loss on drying and it was found to be 9.71%. Ash values indicate the presence of mineral content and purity and it should be less than 10%. Ash value obtained by the analysis of our extract indicates that contamination is very low in our sample. This characteristic make the sample a promising candidate future pharmacological and phytochemical studies, where high pirity is essential for accurate and reliable result.

The foaming ability of plant material and their extract is measured in terms of foaming index (Modak, 2016). Foaming index of 100 and more indicates the presence of saponins and our plant material contains saponins. Saponins act as chemical barrier or shield in the plant defense system to counter pathogens and herbivores (Augustin, Kuzina, Anderson and Bak, 2011). Swelling index of 3ml indicates the mucilage content. The pH of grona extract in solution was 4.77 which is slightly acidic in nature.

Grona triflora extract is used for phytochemical screening. Whole plant is used for extraction. Phytochemicals like Saponins, Tannins, Terpenoids, alkaloids, Flavanoids and Glycosides were present in the extract. All these phytoconstituents were reported to possess a number of biological activities.

Anti-inflammation is the process of minimizing or preventing the body's inflammatory response, which normally occurs due to injury, infection, or harmful stimuli. Although inflammation helps protect the body, excessive or prolonged inflammation can harm tissues and lead to chronic diseases. Anti-inflammatory effects include reducing swelling, pain, redness, and heat, as well as preventing tissue damage. Compounds with these effects, known as anti-inflammatory agents, may be natural (such as flavonoids, turmeric, and ginger) or synthetic (such as ibuprofen and corticosteroids). These agents function by interfering with pathways or chemicals that cause inflammation.

The plant *grona triflora* was subjected to anti-inflammatory analysis and the presence of anti-inflammatory agents indicates that the plant may have the potential to reduce or prevent inflammation in the body. The anti-inflammatory analysis compares the effects of a standard drug and a plant sample at concentrations from 20 to 100 µg/mL based on their percentage of inhibition. Both show a

Table 5. Regression analysis

Assay	Regression values (R^2) Of plant	Regression values (R^2) Of standard	Regression equation of plant	Regression equation of standard
Albumin Denaturation assay	0.9125	0.9872	0.6644x+31.334	Y=1.0436x-10

steady increase in inhibition as the dose increases. The standard starts with 14.36% inhibition at 20 µg/mL and reaches 93.33% at 100 µg/mL. In contrast, the plant sample shows higher inhibition at all levels, starting at 36.72% and reaching 94.37%..This suggests that the plant could be useful in treating or managing inflammatory conditions such as arthritis, infections, or skin irritations. It also supports the plant's possible therapeutic value and may guide further research for the development of natural medicines or supplements.

The gas chromatogram illustrates the elution of different compounds over time, with the peak heights reflecting their relative concentrations in the plant extract. As each compound is eluted, the mass spectrometer analyzes it to determine its chemical structure and identity. Larger molecules break down into smaller fragments, producing a unique pattern of peaks at specific mass-to-charge (m/z) ratios. These mass spectra serve as a distinctive fingerprint for each compound, allowing for identification by comparison with reference libraries (Table 5 and figure 3)

The phyto components present in the extract of *grona triflora* were identified by GC-MS analysis, GC-MS running time being 30 min. The present study on GC-MS revealed the presence of 70 active compounds.(Table 6). Of the major constituents identified, D-Fructose, 3-O-methyl- is present in highest quantity followed by 9,12-Octadecadienoic acid (Z,Z)- , .beta.-D-Mannofuranoside, farnesy, n-Hexadecanoic acid , 1-Butanol, 3-methyl-, formate , gamma.-Sitosterol , Octadecanoic acid , 9,12-Octadecadienoic acid (Z,Z)-, methyl ester , Undec-10-ynoic acid, isobutyl ester ,stigmasterol, Vitamin E , Distearin . The constituents identified with lowest quantity is Di(1-methyl-1-silacyclobutyl) amine.

The study indicates that *grona triflora*, contains a wide range of medicinally valuable phytochemicals. These bioactive compounds can be effectively harnessed for therapeutic use through proper isolation and extraction techniques. They grow in almost all areas for medicinal purposes. Further analysis on *Grona triflora* may help to explore new areas like receptor binding, gene expression modulation or enzyme inhibition.

Conclusion

The present study on the pharmacognostic evaluation of *Grona triflora* offers essential data for the accurate identification of this medicinal plant. Along with this, the analysis of its physicochemical and phytochemical characteristics, as well as the development of analytical methods, contributes to the potential use of the plant in crude drug formulation. These findings may serve as reference standards for assessing the quality and authenticity of *Grona triflora* in future research and therapeutic applications.

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