

**IDENTIFICATION OF BIOACTIVE COMPOUNDS PRESENT IN
DICHLOROMETHANE (DCM) FRACTION OF *URARIA PICTA* USING
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)**

BY

**FAVOUR UKAMAKA ILOBA
LSC1906523**

**DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY**

**IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE
DEGREE OF BACHELOR OF SCIENCE (B.SC) IN BIOCHEMISTRY**

APRIL, 2024

DEDICATION

This project work is dedicated to God Almighty who has made this project successful. Also to my beloved parents (Mr. and Mrs. Nwobi) and to my loving brother, Iloba Emeka Daniel for their support, unconditional love and financial support throughout my project work.

CERTIFICATION

We certify that this project was carried out by **FAVOUR UKAMA ILOBA** of the department of Biochemistry, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. Under the guidance of Mrs. D. Kolawole. In partial fulfillment of the award of Bachelor of life science (B.Sc.) degree in Biochemistry.

.....
DR. (MRS.) O.R. USIFO
(Project Supervisor)

.....
DATE

.....
DR. S.I. OJEABURU
(Project Co-ordinator)

.....
DATE

.....
PROF. E. CHUKWU ONYENEKE
(Head of Department)

.....
DATE

ACKNOWLEDGEMENT

External gratitude goes to God Almighty, the creator and provider of life, He is the giver of energy that sustained me during this project work. My special gratitude goes to the Head of Department Prof. E.C. Onyeneke and to my wonderful project supervisor Dr. (Mrs.) O.R. Usifo for her encouragement, motivation and diligence toward this research work.

My profound gratitude goes to my parents Mr. and Mrs. Nwobi and my loving brother Iloba Emeke Daniel for their love, support and care throughout my academic years in school and how they provided everything I needed for the completion of this research work, God bless you. More strength and power to your elbow.

To all my lecturers and technologist of this department whom have imparted great knowledge and given good advice to help me academically. Lastly, I want to thank my colleagues and friends for their support and encouragement in one way or the other during the course of this study.

TABLE OF CONTENT

TITLE PAGE	i
DEDICATION.....	ii
CERTIFICATION.....	iv
ACKNOWLEDGEMENT.....	v
TABLE OF CONTENT.....	vi
ABSTRACT	viii
CHAPTER ONE	
1.1. Introduction	1
1.2. Aim and Objective	3
CHAPTER TWO	
2.1. Literature Review	4
2.2. Description of <i>Uraria picta</i>	5
2.3. Botanical Aspects.....	6
2.3.1. Morphology	6
2.4. Ethnomedical/Therapeutic Uses of <i>Uraria picta</i>	8
2.5. Phytochemistry	9
2.6. Pharmacology	9
2.7. Hepatoprotective Effects	10

2.8. Anti-acaricidal Activity	10
2.9. Antimicrobial Efficacy	10
2.10. Antioxidant Activity	10
2.11. Cancer Activity	11
2.12. Protective Effect	12
2.13. Antidiabetic Activity	12
2.14. Bioactive Compounds	13
2.15. Classification of Bioactive Compounds	14
2.16. Fractionation	20
2.17. Identification and Characterization	23
 CHAPTER THREE	
3.0. Materials and Method	27
3.1. Equipment and Apparatus	27
3.1.2. Chemical and Reagents	27
3.1.3. Plant Collection and Identification	27
3.1.4. Plant Preparation and Extraction	27
3.1.5. Preparation of Stock Solution	28
3.2. High performance liquid Performace Chromatography	29
3.2.1. Definition	29

3.2.2. Extraction	29
3.2.3. Analysis	29
3.2.4. Calculation	30
3.2.5. HPLC Programming	30
CHAPTER FOUR	
4.0. Result	31
CHAPTER FIVE	
5.1. Discussion and Conclusion	33
5.2. Conclusion	36
APPENDIX.....	37
REFERENCE.....	39

ABSTRACT

Bioactive compounds are important nutritional components that are present in small amount in fruits and vegetable and are known for their various behavioral, immunological and physiological health benefit. This research utilized HPLC (High Performance Liquid Chromatography) analytical method, to identify and quantify the phytochemicals and bioactive compounds present in the Dichloromethane fraction of extract of *Uraria picta*. Analysis by HPLC revealed Six prominents compounds which include Ammodendrine (22.0694 ug/ml), Cyanogenic glycoside (20.1936 ug/ml), Spartein (15.9760 ug/ml), Tannin (12.9619ug/ml), Sapogenin (10.9960 ug/ml) and proanthocyanidine(10.4914 ug/ml) with antioxidant, Anticancer and anti inflammatory potentials. These bioactive compounds have been reported to possess multiple therapeutic activities, which explain the use of *Uraria picta* leaves in traditional medicine to treat numerous diseases. This study suggests that *Uraria picta* leaves could be exploited for the development of new therapeutic molecules.

CHAPTER ONE

1.1. INTRODUCTION

Bioactive compounds are important nutritional components that are present in small amount in fruits and vegetable and are known for their various behavioral, immunological and physiological health benefit. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos *et al.*, 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyar *et al.*, 2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim.

Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate

and long-term side effects and their outcomes are measured before the drug is widely applied to patients. Plants are known to be capable of biosynthesizing a myriad of primary and secondary metabolites, the latter being important natural products with therapeutic potential important to human health (Twaij and Hasan, 2022). The use of plants as medicine dates to the prehistoric era and an account of the same is there in ancient manuscripts of India, China, Egypt, America, Europe, Arab countries, and African countries (Gurib-Fakim, 2006). About 80% of the global population depends on plant-based medicaments. The International Union for Conservation of Nature and the World Wildlife Fund have suggested that there are 50,000–80,000 flowering plant species used for therapeutic purposes globally. India, China, Sri Lanka, Thailand and USA use 20%, 18.9%, 16.6%, 15.5% and 11.8%, respectively, of total plant species for medicinal purpose (Sengupta *et al.*, 2018). In the United States, about 79% of the top 150 prescribed medicines are derived from natural sources, with plant-based medicines accounting for about 74% (Naguib, 2011). In developing countries like India and China, phytodrugs comprise 25% and 80%, respectively of the total drugs (Sengupta *et al.*, 2018).

Over the years, the side effects and residual toxicity associated with synthetic medicines have resulted in a global switch towards alternative therapies for the treatment of ailments. Viewing the importance of medicinal plants, the World Health Organization (WHO) in 1978 started collecting various information related to the use of medicinal plants. Since then, the organization has been proactive in this area and came out with a Traditional Medicine Strategy for 2014–2023 (WHO, 2014) with an aim to integrate Traditional and Complementary Medicine into the Healthcare system. Thus, there is an evident upsurge in the research taking lead from traditional systems of medicines to identify plant bioactives as a potential source of new drugs. Moreover, artemisinin (Gao *et al.*, 2020) and the global inclination towards plant-based remedies in the

recent incidence of COVID-19 have given the right impetus to this field of research. One of the major concerns with the medicinal plant is their over-exploitation for commercial purposes. A rather conservative estimate shows that the loss of plant species is 100–1000 times higher than the natural rate of extinction (Pimm *et al.*, 1995). In order to cater to the global market, the plants are being harvested at a very rapid rate and in a substantial volume from the wild. Deforestation and change in the use of land have added to this loss. According to Bentley (2010) about 15,000 species are on the verge of extinction due to loss of their habitat and overharvesting. There is a need for an approach that serves a dual purpose (i.e., conserve plants and provide the raw material required to supplement the ever-growing demand for medicinal uses). Propagation via tissue culture can be an effective alternative to solve the aforesaid problem. The tissue culture system can be used to have clonal propagation of elite plants and it can also be manipulated/modified to have an enhanced yield of the metabolite of interest (Vats and Kamal, 2013; Vats and Mehra, 2020).

1.2 AIM AND OBJECTIVE:

The aim of the study was to identify and quantify the bioactive compounds present in dichloromethane (DCM) fraction of *Uraria picta* using High Performance Liquid Chromatography (HPLC).

CHAPTER TWO

2.1. LITERATURE REVIEW

The bioactive compounds or phytochemicals are important nutritional components that are present in little amounts in fruits and vegetables and are well known for their various behavioral, immunological and physiological health. Many antioxidant compounds can be found in fruits and vegetables including phenolics, carotenoids, anthocyanins, and tocopherols (Jakubowski, 1997). Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases (Naczek, 2006). Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini, 2004). Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots, 2008). Various studies have shown that many plants are rich source of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, saponin, tannins, and lignins, found in plants, all act as antioxidants (Suffredini, 2004). The consumption of fruits and vegetables has been linked with several health benefits, a result of medicinal properties and high nutritional value (Valko, 2006). Antioxidants control and reduce the oxidative damage in foods by delaying or inhibiting oxidation caused by reactive oxygen species (ROS), ultimately increasing the shelf-life and quality of these foods (Ames, 1993). Beta carotene, ascorbic acid, and many phenolics play dynamic roles in delaying aging, reducing inflammation, and preventing certain cancers (Duthie, 1996). *Uraria picta* being a leguminous plants are one of those plants whose bioactive components possess high medicinal and pharmaceutical importance, thus the need to study and identify its biochemical components using a separation technique

(HPLC) for better understanding. In Nigeria, *Uraria picta* are widely utilized in herbal medicine .(Micheal and fowler, 2006). The biological and effects of these plants include anti-cancer, anti- inflammatory, Anti-acaricidal activity and among others.

2.2. Description of *Uraria picta*

Uraria picta , belong to the Leguminosa family ,it is commonly called “Wizardry” or “Slight-of-hand. It is also found throughout India and almost all places in Bangladesh, Sri Lanka, tropical Africa, the Malaysian Islands, the Philippines, Australia, Africa, and Asia (Sastri and Ohas.hi, 1962). Especially in some diseases of traditional medicine, it is a very important plant, compared to many other plants, in terms of its usefulness and effectiveness. The suffruticose plant is vertical or fertile, 1.5 m high and is often found in dry grasslands, bare lands and forests. It is one of the key ingredients of “Dashmoolarista,” the main Ayurvedic medicine in the Indian medical system, made from the roots of 10 medicinal plants and used for treating general fatigue, mouth ulcers and various gynecological diseases. The formulation Dashmula contains ten roots of medicinal plants: Gambhari (*Gmelina arborea*), Bilva (*Aegle marmelos*), Agnimantha (*Premna integrifolia*), Shoynaka (*Oroxylum indicum*), Patla (*Sterospermum suaveolens*), Shalaparni (*Desmodium gangeticum*), Kantkari (*Solanum xanthocarpum*), Gokshura (*Tribulus terrestris*), and Brahati (*Solanum indicum*) (Kirtikar and Yusuf 1935). Dashmool is also used as an essential ingredient in the manufacture of more than 109 drug formulations (Pathak and Saxena, 2014). It is also used in other Ayurvedic formulations such as Abana, Amritarishta, Angamardana prashamana kashaya churna, Dashamoola carving, Vyaghri carving, Madhyama Narayana carving, Dashmularishta, and Shira Shuladi vajra Ras (Sastry, 199). Almost all parts of *Uraria picta* have therapeutic value and are used in the medical system of India to treat fatigue, oral ulcers, and various gynecological diseases (Yadav, 2009). *Echis carinatus* is also known as

an antiseptic to cure snake (Kirotkar, 1935) and fractures (Guarav, 2008). It contains antiseptic (Khare, 2007), antimicrobial (Rahman, 2007), acaricide (Igboechi, 1989), antiulcerogenic (Manonmani, 1995), antihypodynamic (Occhiuto, 1994), and antipulmonary hypertension (Occhiuto, 1990) and partial vasodilation (Yadav and Pathak, 2009). In addition to its use in the traditional pharmacological system, it is widely used in the pharmaceutical and pharmaceutical industries to make various formulations. The quality and efficacy of a plant depends on rhoifolin (apigenin-7-o-neoesperidoside), a biologically active compound used as a chemical marker, and rhoifolin, which has a high environmental impact (Saxena, 2016)

2.3. Botanical Aspects

2.3.1. Morphology

Uraria picta is a straight tree between 1 and 2.5 m tall. At the bottom are wooden stems. The leaves are composed of 2–5 leaflets and pinnate pairs. In the vegetation phase, the pairs of leaves appear without the final leaves. The lower leaves are oval, 2–8 cm long and 2–3 cm wide; the upper leaves are 7–25 cm, 5–25 mm wide, and oval-lanceolate. The number of inflorescence flowers is between 35 and 75 (it is 10–70 cm long on a stem 0–5 cm long), usually in dense clusters like thorns that are pink or purple 1.5 m long. There are long clusters and terminals. The bracts are the highest at the base and top. The fruits are between 2 and 6 seeds and the parts are almost separated. The fruits are hairless, distinctive, and gray. The seeds are light brown, tall, and about 2.5×1.5 mm.

Plant growth habit: Annual woody erect

Soil: Loam to clay loam

Plant size: 1–2.5 m height

Leaf: $1.4\text{--}7.2 \times 0.6\text{--}3$ cm, elliptic-oblong

Flowering season: July to September

Flower: Bright reddish purple

Fruit shape and size: 0.4–1.2 cm long

Fruit color: Gray

Seed: Light brown, oblong, 2.5 × 1.5 mm

Fruit season: December to January

Taxonomical classification:

Kingdom: Plantae

Subkingdom: Viridiplantae

Superorder: Rosanae

Order: Fabales

Class: Magnoliopsida

Subclass: Rosidae

Family: Leguminosae

Sub-family: Fabaceae

Genus: *Uraria* • Species: *Picta* –(Groom and Saxena,2012)

Vernacular names:

Sanskrit: Citraparni, Kalasi, Dhavani, Prishniparni, Galvanina

Hindi: Pithava, Dabra

Marathi: Pithava, Prishniparni

Tamil: Oppai

Telugu: Murele Honne, Andale home, Prushniparni

Gujarati: Pithava

Bengali: Salpani, Chhalani, Chakule

Kannada: Kolakuponna

Malayalam: Oril

Panjabi: Detedarnee

Oriya: Prushnipamee, Shankar Jata (Committee,2004)

2.4. Ethnomedical /Therapeutic Uses of *Uraria picta*

Uraria picta is prescribed for cough, chills, and fever. The antiseptic leaves are well thought out and used to treat gonorrhoea. The roots and pods are used to treat infantile rectal prolapse. The capsules are used to treat oral pain in children. It is used to treat urinary tract diseases, tumors, and inflammation, and breathing problems. Its paste mixed with water is used as a remedy for snake bites. It is the Ayurvedic medicine of the Indian system of medicine for the treatment of general fatigue. It is an antioxidant, analgesic, and anti-inflammatory drug in medicine (Parrota and Ved, 2007). The herb is traditionally used as an antipyretic, diuretic, astringent (used for irritable bowel syndrome, diarrhoea, and dysentery) against colds, diuretics, anthelmintics, laxatives, and tonic nerves. As in China, where *Uraria picta* is used as a popular remedy, it is mainly used to treat fever, neutralize toxins, relieve pain, stimulate blood circulation, relieve cough, and improve breathing. (Charaka and Sushruta, 1970) prescribed the entire plant, internally in prescriptions, in misperistalsis, diarrhoea, dysentery, cough, consumption, respiratory diseases, abdominal glands, and fever from the inside of the whole herb; relieve asthma attacks as an ingredient in a drink to increase breast milk.

2.5. PHYTOCHEMISTRY

The plant is said to contain alkaloids, flavonoids, steroids, terpenoids, phenols, and saponins, all of which are components of plants. The tannins were not in the stem and roots, and the glycosides in the internal organs were in the roots (Saxena, 2014) Various significant compounds secluded from the root bark of *Uraria picta* are 5,7-dihydroxy-2'-methoxy-3',4'-methylenedioxyisoflavanone and 4',5-dihydroxy-2',3'-dimethoxy-7-(5-hydroxyoxychromen-7yl)-isoflavanone (Rahman, 2007), stigmasta-4, 22-diene-3-one, β -Sitosterol, and lupeol. Recently, a flavonoid rhoifolin (Apigenin-7-o-neohesperidoside) has been isolated from this plant (Sexana,2014)

2.6. PHARMACOLOGY

Anti-inflammatory Singh, 2017, showed significant activity against both inflammatory models. Extraction of methanol from the *Uraria picta* roots reduced the inflammation of the treated egg albumin and formalin in a dosedependent manner(Singh,2017). Naik and Krishnamurthy, in 2018, performed anti-inflammatory activities against the whole plant against leg edema caused by carrageenan of *Uraria picta* methanol extract in albino rats. Methanol extracted from *Uraria picta* was extracted at weight doses of 200– 400 mg/kg. The measure of inhibition of the extracts was compared with a standard reference drug indomethacin. This study provides information on pharmacological evidence showing that *Uraria picta* can be used as an anti-inflammatory agent (Nailk,2018). Regarding anti-inflammatory activity, Olufemi *et al.*, in 2016, the *Uraria picta* leaf ethanol initiative significantly inhibited edema depending on the inflammation caused by carrageenan and the formation of granuloma caused by Cotton pinch in mice. It has anti-inflammatory activities due to stabilization of the lysosomal membrane of COX-2 (Olufemi and Ghildiyal, 2016)

2.7. Hepatoprotective Effects

Singh, in 2017, tested liver damage caused by PCM 2000 mg/kg in mice to increase blood levels of the enzyme alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) in mice. Administration of methanol extract from *Uraria picta* roots reduced ALT, ALP, and AST enzyme levels, equivalent to the standard drug silymarin (Singh and Arka,2012)

2.8. Anti-acaricidal Activity

Igboechi et al. examined the acaricidal activity of extracts of *Uraria picta* in 1989. In total methanol extraction has been found to be 21 times more effective than acaricide than water extract. This aspect of efficacy clearly shows that the acaricidal principles of *Uraria picta* are better removed from water with alcoholic solvents (Igboechi and Srilakshmi ,1998)

2.9. Antimicrobial Efficacy

Osazuwa and Igboechi, in 1988, carried out antimicrobial activity of two chemicals isolated from *Uraria picta* leaves. Isolation had significant static or bactericidal and fungistatic or fungicidal activity. These effects have been demonstrated in numerous microorganisms. The results of the study provide scientific support for the timely application of *Uraria picta* leaves to treat skin diseases and to accelerate the healing of fractures (Rahman and Osazuwa,1998)

2.10. Antioxidant activity

Patel *et al.*,(2011), approaches the free radical elimination potential of *Uraria picta* ethanol extract. Several in vitro models have been studied for their radical elimination properties, namely, DPPH radical scavenger test, ABTS radical scavenger test, O-phenanthroline test, lipid

peroxidation test, superoxide capture test, non-enzymatic hemoglobin glycosylation test, and total antioxidant. The total antioxidant capacity of the ethanol extract from *Uraria picta* (10 mg/ml) corresponds to 63.31 mg/ml ascorbic acid. It showed 84.89% inhibition in the nonenzymatic hemoglobin glycosylation test. In summary, significant antioxidant activity has been associated with the presence of phenol derivatives, flavonoids, sterols, and terpenes (Patel, 2011]. Mohan *et al.*, 2019, determined the free radical elimination activity from the leaves, stems, and roots of *Uraria picta*. Antioxidant activity was determined using the DPPH method to eliminate free radicals. It was found that ethanolic extracts and aqueous root extract from leaves and stems contain the lowest IC₅₀ and hence the highest antioxidant activity. Based on the results, it can be concluded that the most promising antioxidant activity was demonstrated due to the presence of various extracts from the leaves, stems, and roots of *Uraria picta*, methanolic extracts from leaves and stems, and aqueous extracts from the roots of phenols and flavonoid compounds (Saxena and Mohan, 2014).

2.11.Cancer Activity

In 2013, Eldahshan studied rhoifolin as an antitumor activity that is almost comparable to vinblastine, tested against human cell laryngeal occult carcinoma (Hep 2) and human cervical carcinoma (HeLa) cell lines. Promising activities have also been achieved against hepatocellular carcinoma (Hep G2), large intestine (HCT-116), and human fetal lung fibroblasts (MRC-5).

A special effect of rhoifolin was the absence of cytotoxic activity against normal healthy cells (Vero cells), indicating the high selectivity of this compound (Agrawal and Eldahshan, 2018)

2.12. Protective effect

Kale *et al.*, in 2012, overuse of NSAIDs (anti-inflammatory steroids) resulted in nephrotoxicity, such as paracetamol, liver necrosis, and renal failure, occurring in approximately 1–2% of patients with acetaminophen overdose. Treatment with *Uraria picta* gold extract containing polyphenolic compounds and carbohydrates can significantly reduce urine urea levels, increase serum creatinine levels and urine creatinine with the paracetamol group. The stimulating activity of the summary may be due to its ability to activate antioxidant enzymes. The results suggest that it may be used as a new therapeutic agent that is not suitable for adapting the water extract of *Uraria picta* (Kale *et al.*.,2012)

2.13. Antidiabetic Activity

Fatokun *et al.*, 2012, studied the activity of oral glucose tolerance of *Uraria picta* (leaves) in a model animal fed on a high-calorie diet to induce Type II diabetes. Sprague-Dawley 120 male rats were divided into two main groups. One group received a normal rat diet and the other group a high-calorie diet for 4 months. Raw herbal extracts were made by boiling, pressing, drying, and heating using traditional methods of sweetening. The untreated group and the rat group treated with metformin were used as controls and comparators, respectively. Each animal received different doses of oral herbal extracts at 3 weeks. Blood was taken from all rats before the analysis of glucose concentration. During this period, the animals were weighed weekly and fed every 3 days. After the dosing period, an oral glucose tolerance test was performed and blood samples were taken empty on the stomach for 0, 30, 60, and 120 min, and glucose concentrations were analyzed. *Uraria picta* showed a marked difference in the effect of reducing plasma glucose in rats fed a normal diet.

DIAGRAM OF *URARIA PICTA*



Source: Mane *et al.*, (2011)

2.14.BIOACTIVE COMPOUNDS

The bioactive compounds or phytochemicals are important nutritional components that are present universally in little amounts in fruits and vegetables and are well known for their various behavioural, immunological and physiological health benefits. A large number of bioactive compounds have been identified which are classified according to their chemical structures and functions. Some of the examples of bioactive compounds are polyphenols, flavonoids, lycopene, carotenoids, anthocyanin, tannins, terpenoids, saponins, taurine, phytoestrogens, etc.

2.15. CLASSIFICATION OF BIOACTIVE COMPOUNDS

Phenolic compounds, Alkaloids, Terpenes and Terpenoids, are the main classes of bioactive compounds (Croteau *et al.*, 2000). Fig 1 indicates the detailed classification of bioactive compounds present in plant products.

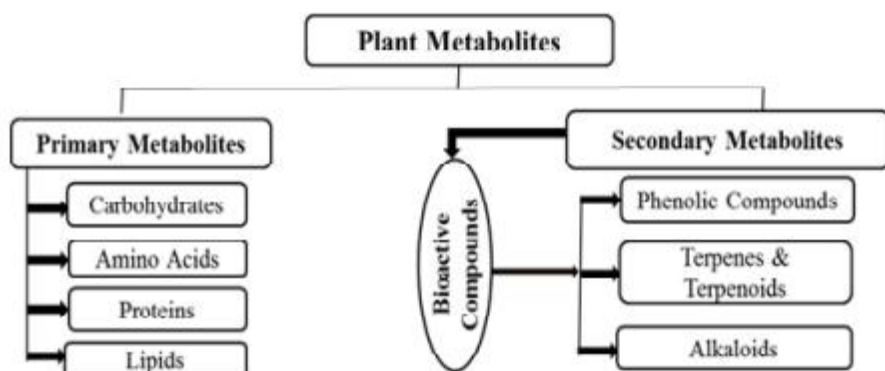
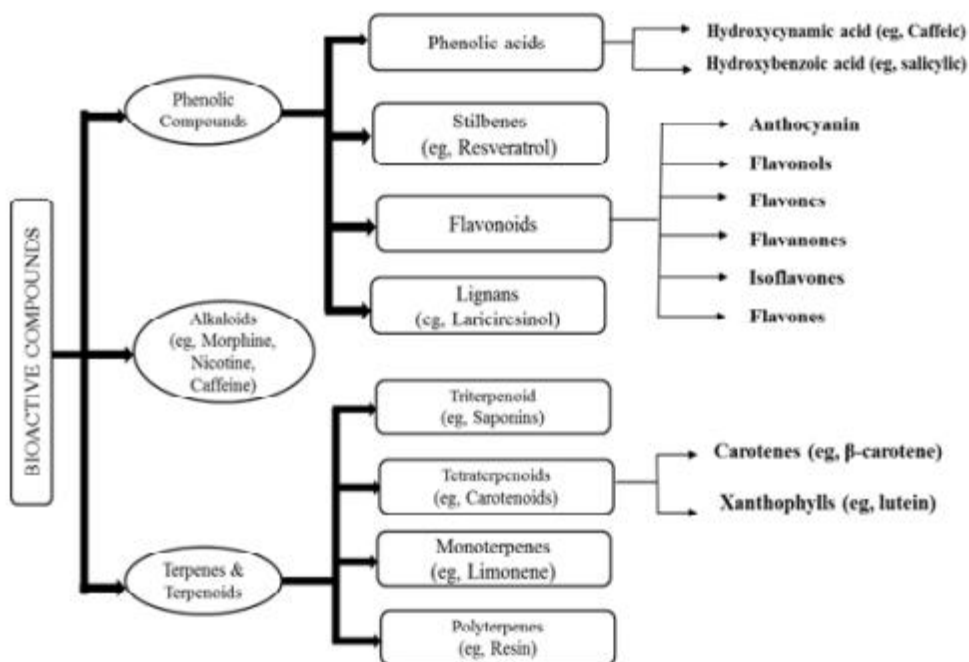


Fig 1: Classification of plant metabolites.



Source: Croteau *et al.*, (2000)

Phenolic Compounds

Phenolic compounds, present naturally in plant products, are an essential part of the human diet. These compounds have an aromatic ring that bears one or more hydroxyl groups and their structures vary from a simple phenolic molecule to a complex high molecular weight polymer. These compounds have a large number of physiological properties such as antioxidant, anti-allergenic, anti-inflammatory, anti-atherogenic, anti-microbial and cardioprotective (Balasundram *et al.*, 2006). These compounds, most widely found groups of phytochemicals, find considerable importance in the physiological and morphological activities in plants. They play a vital role in their growth and reproduction. They also provide protection against predators and pathogens apart from providing colour and sensory characteristics to fruits and vegetables (Bravo, 1998; Alasalvar *et al.*, 2001).

Flavonoids:

Flavonoids are low molecular weight compounds, made up of fifteen carbon atoms and are arranged in C₆-C₃-C₆ configuration. Structurally they have two aromatic rings joined by a 3-carbon bridge. They are further classified into flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanin. Flavonoids are an important source of antioxidants because of their high redox potential that enables them to act as reducing agents. The consumption of flavonoids in large amounts helps in preventing cancer and heart diseases (Ignat *et al.*, 2011)

Anthocyanins:

Anthocyanins belong to the class of flavonoids and they are water-soluble vacuolar pigments that may appear as purple, red or in blue colours depending on their pH. They occur in all plant tissues, including fruits, stems, leaves, flowers and roots (Ignat *et al.*, 2011). Anthocyanins

donate hydrogen to highly reactive radicals and thus act as an antioxidant and prevent further radical formation.

These are coloured compounds and can be used in place of synthetic dyes for colouring foods. Since they are water-soluble, that allows them to incorporate into aqueous food systems easily; thus, they are helpful in additional health benefits to such dyed food products (Kammerer *et al.*, 2004)

Alkaloids:

Alkaloids are secondary metabolites which are defined as active compounds, mainly composed of nitrogen. They are formed from one of the few amino acids such as lysine, tyrosine and tryptophan. Around 150 families of more than 12000 alkaloids have been identified in plants and it is estimated that around twenty percent of the ‘species of flowering plant’ contain alkaloids. Alkaloids are generally present in plant in the form of salts of organic acids such as acetic, malic, lactic, citric, oxalic, tartaric, tannic and other acids. Few weak alkaloids such as nicotine freely exist in nature. Examples of alkaloids are also present as glycosides of sugar such as glucose, rhamnose and galactose (Zotchev, 2013).

Uses of Alkaloid in plants:

1. Defense against Herbivores:

- i. Toxicity: Many alkaloids are toxic to herbivores, acting as chemical defenses that deter grazing animals and insects from consuming plant tissues.
- ii. Repellent Properties: Alkaloids like nicotine and quinine can serve as insect repellents, protecting plants from pest infestations.

2. Allelopathic Interactions:

- i. Weed Suppression: Alkaloids released by plants can inhibit the growth of neighboring plants and act as allelochemicals, contributing to weed suppression and competition.

3. Pollinator Attraction:

- i. Nectar Composition: Alkaloids present in floral nectar serve as attractants for pollinators like bees, butterflies, and hummingbirds, enhancing the plant's reproductive success.

4. Medicinal and Therapeutic Uses:

- i. Traditional Medicine: Many alkaloids have medicinal properties and are used in the treatment of various health conditions in humans and animals.
- ii. Drug Development: Alkaloids serve as valuable sources for developing pharmaceutical drugs, such as pain relievers, anti-inflammatories, and anticancer agents

Terpenes and Terpenoids

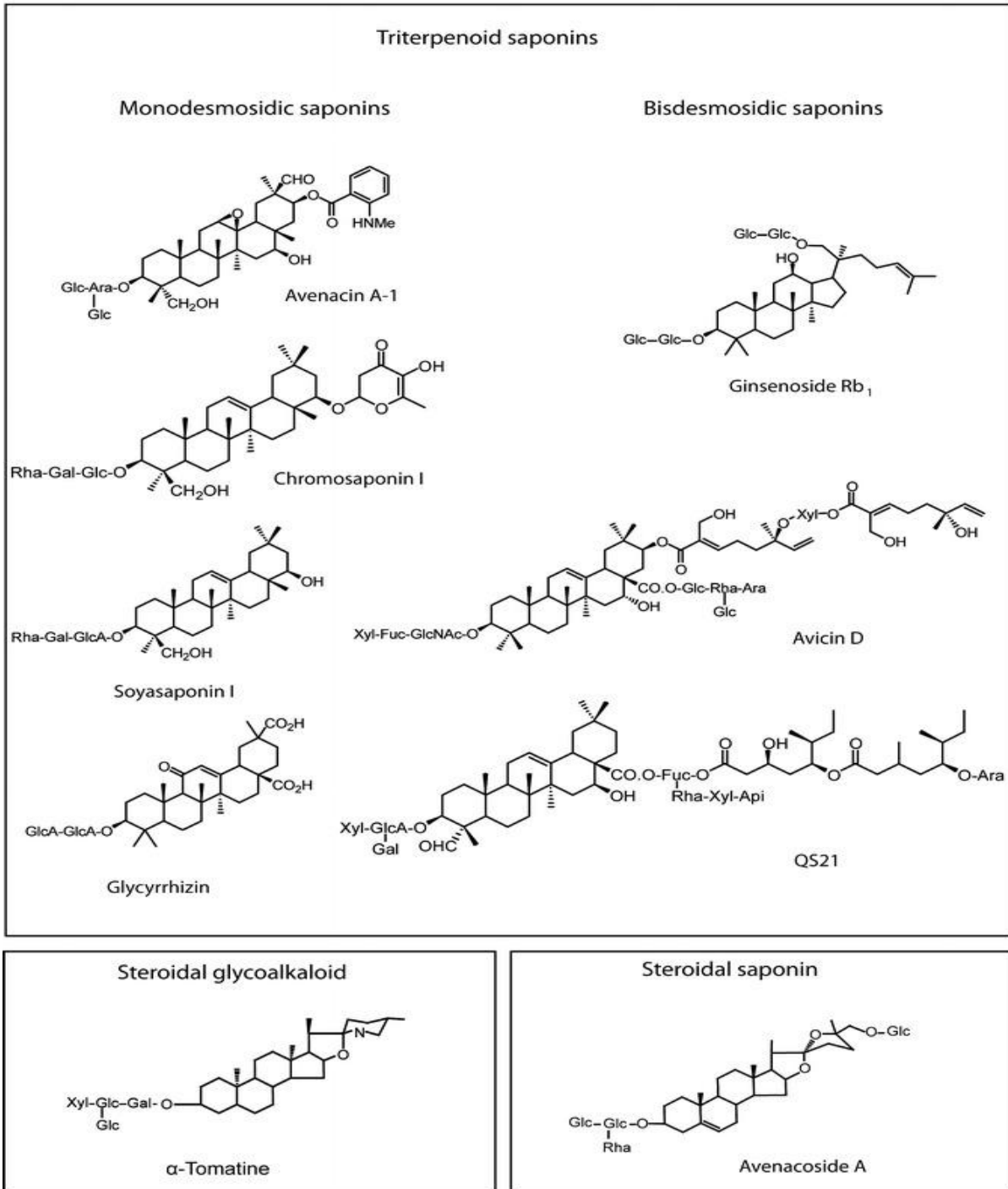
Carotenoids:

Carotenoids are fat-soluble pigments present in large amounts in some fruits and vegetables. The four primary sources of carotenoids in human diets are lycopene, β cryptoxanthin, β -carotene and lutein, but as many as 750 members of this family are identified (Degrou *et al.*, 2013). They are beneficial to our health as these compounds are converted into vitamin A or retinol. β -carotene is the essential provitamin A with the highest activity, but when consumed as a separate supplement can have harmful effects

Saponins:

Saponins are glycosides of triterpenes and steroids . Steroidal glycoalkaloids are sometimes also referred to as saponins. The triterpene and steroid backbones are both derived from the mevalonic acid pathway, the common precursor being 2,3-oxidosqualene . The name “saponin” derives from the soap-like properties of these compounds. The highly polar sugar moieties together with the non-polar triterpene or sterol backbones result in a highly amphipathic compound. Hence, these compounds produce stable foams, a feature often associated with aqueous extracts from saponin-accumulating plants (Hostettmann and Marston, 1995). Indeed, the names of some plants originate from this property, such as soapwort (*Saponaria officinalis*), which was historically used as a source of detergent.

Structure of Saponin



Source: Osbourn *et al.*, (2011)

2.16. Fractionation

Fractionation is a process of separation of plant extracts into various fractions. It further segregates the fractions into portions comprising a number of compounds. The process continues until pure compound is isolated.(Doughari *et al.*, 2012) When several solvents are required for the fractionation, they should be added according to the order of increasing polarity. Fractionation techniques are basically classified into physical or chemical method.

Chemical methods:

This extraction method is based on the type of functional groups possessed by a compound in the given mixture. Separation or purification can be achieved by chemical reactions using appropriate reagents.(Doughari, 2012)

Physical methods:

Physical methods used in separation of compounds from mixtures include separation funnel method, chromatographic techniques, fractional distillation, fractional crystallization, fractional liberation, and sublimation.(Doughari, 2012)

- a) **Separation funnel method.** When the following different solvents (n-hexane, chloroform, acetone, dicloromethane and n-butanol) are selected, fractionation begins by moistening or complete dissolution of crude extract with 300mL of water. This is followed by transfer into a separating funnel, shaken, and allowed to settle. Furthermore, to 500mL of dichloromethane, the least polar solvent was added and shaken. The content can settle, and the bottom of the separating funnel opened to remove the aqueous layer. The remaining content in the separating funnel was poured into a clean container to get

dichloromethane fraction.(Igle and Sasidharan ,2017) Equal volume of dichloromethane was added again, shaken, and separated. The addition continued until after adding dichloromethane and shaken no reasonable quantity of extract appeared to move into the DCM portion Similar cycle was performed for chloroform, acetone, n-butanol to get chloroform, acetone, and n-butanol fractions. The remaining portion left after the fractionation is termed as residual aqueous fraction (RAF) as the crude extract was first dissolved in water. (Igle *et al.*, 2017)

b) **Fractional distillation.** This is a process of separating or purifying compounds from a mixture. It is usually used in separation of hydrocarbons such as crude oil, citral, and eucalyptol. Purification is achieved based on the differences in their boiling points.

Fractional distillation apparatus is constructed in such a manner that when heat is applied each compound will evaporate and separates at its boiling point. Consequently, each compound fractionated will condense and collected as a separate entity through several siphons attached to fractional distillation apparatus. (Doughari, 2012)

c) **Fractional crystallization.** Large numbers of compounds that exist naturally in plant extracts are crystal in nature. Separation is achieved via formation of crystals during concentration of an extract using heat or refrigeration. .(Doughari, 2012)

d) **Fractional liberation.** This method is suitable for separating compounds that can easily form precipitate from the mixture. The precipitate is usually formed by changing the compounds into their salt form. Fractional liberation is commonly applicable in purification cinnamon alkaloids. .(Doughari, 2012)

e) **Sublimation.** This method involves changing from solid to gaseous state without passing through liquid state. Substances such as camphor and volatile oils when heated get separated and converted directly into gas. (Doughari, 2012)

f) **Chromatographic techniques.** These are special techniques used in separation of compounds from mixtures based on their size, shape, and charge. The concept of chromatography involves the use of mobile phase, which is the solvent of extraction and the stationary phase such as silica gel and sephadex mixed with a calcium sulfate as a binder.(Ighle and Heftman *et al.*,2017) Silica gel is used for parting amino acids, sugars, fatty acids, lipids, and alkaloids. Sephadex is applicable in isolation of proteins and amino acids. Aluminum is useful in separation of vitamins, carotenes, phenols, steroids, and alkaloids. Cellulose powder is used in separation of amino acids, food dyes, and alkaloids. Celite is applicable in separation of organic cations and steroids. (Ighle and Doughari, 2017) Various mechanisms were involved in separation compounds using chromatographic techniques, namely, adsorption, partition, affinity, ion exchange, or size exclusion. Chromatographic techniques include PC, TLC, column chromatography (CC), liquid chromatography (LC), GC, and HPLC.(Ighle 2017 and Rimando *et al.*,2001)

Mechanisms of separation in chromatography

- a) **Adsorption chromatography.** Separation is performed based on the interaction between compounds to be separated and the stationary phase. In this case, the stationary phase will pull and remove compounds via hydrophobic, non-covalent Van der Waals forces of attraction. The compound that is loosely bound will first be eluted by the mobile phase.(Heftmann and Sasidharan, 2011)
- b) **Partition chromatography.** Compounds are separated by addition of two or more immiscible solvents in to the mixture of an extract. Each compound will part away from the mixture by dissolving in the portion of solvent where it is soluble. Subsequently, the

immiscible liquids will be separated using separating funnel to obtain the individual compounds. The partition chromatography is otherwise known as liquid/liquid separation.

- c) **Affinity chromatography.** The stationary phase is a ligand positioned in a separating column. The mobile phase applied washed down the compounds that have no affinity for the stationary phase. As such, compounds with high affinity for stationary phase get attracted and separated .(Ighle, 2017)
- d) **Ion exchange chromatography.** The concept of ion exchange is useful in separation of polar compounds based on the type of charge they possessed. As such like charges attract, whereas unlike charges repelled. Like-charge substances attracted to each other and get separated the mixture or extract. .(Ighle, 2017)
- e) **Size exclusion chromatography.** This method considers separating compounds based on their molecular size by application of mesh of different diameters. It is also known as gel filtration or molecular sieving .A smaller size mesh was first applied followed by medium size, and finally larger pores size mesh.

2.17. Identification and Characterization

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains as big challenge for the process of identification and characterization of them. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as Thin-Layer Chromatography (TLC), column chromatography, flash chromatography, Sephardim chromatography and High-Performance Liquid Chromatography (HPLC), should be used to obtain pure compounds (Marthin, 2013).

High Performance Liquid Chromatography

HPLC is a versatile, robust, and widely used technique for the isolation of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture (Cannell and piana,1988) Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants (Fan *et al.*,2006) Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize its properties. The resolving power of HPLC is ideally suited to the rapid processing of such multi component samples on both an analytical and preparative scale (Martin 2005). Several authors describe the use of HPLC for characterization and quantification of secondary metabolites in plant extracts, mainly phenol compounds, steroids, flavonoids, alkaloids (Bolognon and Barbosa, 2004).Reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% of all HPLC separations are carried out in the reversed phase mode

The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass (Ahuja and Prathap ,2013), for example, to identify secondary plant metabolites (Barbosa and Colpo,2012). In addition, the colloquial term used for the mobile phases in reversed phase chromatography is “buffer”.

However, there is little buffering capacity in the mobile phase solutions since they usually contain strong acids at low pH with large concentrations of organic solvents. Adequate buffering capacity should be maintained when working closer to physiological conditions (Prathap,

2013). In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity (Sasidharan, 2011) and also because majority of naturally occurring compounds encountered have some UV absorbance (Cannell, 1998), Phenolics are frequently identified using UV-VIS and Photodiode Array (PDA) detectors at wavelengths 190-380 nm (Khoddami, 2013). The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the Diode Array Detector (DAD) coupled with Mass Spectrometer (MS) (Tsao, 2004). Liquid Chromatography coupled with Mass Spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts. It provides abundant information for structural elucidation of the compounds when tandem Mass Spectrometry (MS) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable (Ye, 2007). The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation (Barbosa, 2014).

The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial

extracting and following a period of maceration, solid material is then removed by decanting off the extract by filtration (Janovik and Sasidharan, 2012). The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns (Goparaju and Ye, 2007)

CHAPTER THREE

3.0. MATERIALS AND METHOD

3.1. Equipment and Apparatus

1. Micro pipette
2. High performance liquid chromatography (Waldbronn, Germany)
3. Freezer drier
4. Rotary evaporator (RE-201D United kingdom)

3.1.2. Chemicals and Reagents

The chemicals and reagents used were of analytical grades. They include dichloromethane solvent, methanol.

3.1.3. Plant Collection and Identification

Uraria picta, also known as Prinshniparni, is a plant with various traditional and potential uses. *Uraria picta* plant leaves were collected from a private farm in Cotonou, Benin Republic. Thereafter, the plant was authenticated by a Botanist, at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

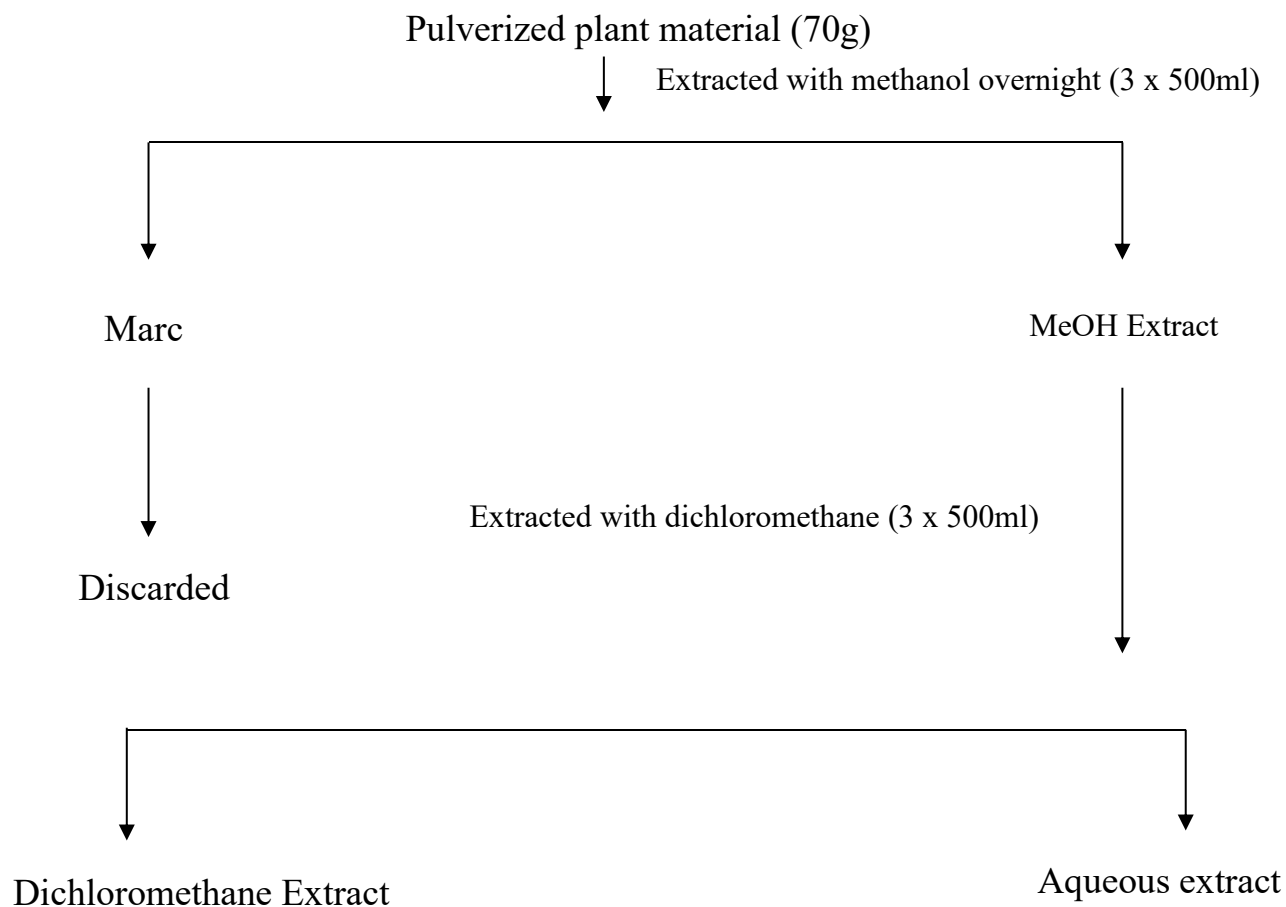
The authenticated plant specimen was later placed in the department's herbarium for safekeeping

3.1.4. Plant Preparation and Extraction

The plant materials were purified, dried in air under shade at room temperature, and crushed or ground into a fine powder. A measured amount of *Uraria picta* (leaves) was dissolved in methanol. This was placed in shaker and left at room temperature for 72 hours(3

days).Afterwards, the filtered liquid was subjected to a rotary evaporator to remove the solvents and concentrate the extract, followed by using a freeze dryer for further concentration.

FRACTIONATION OF PLANT EXTRACTION



3.1.5. Preparation of Stock Solution

A portion of the dichloromethane (DCM) fraction of *Uraria picta* (70g) was measured (weighed) from each of the concentrated fraction into a conical flask which is then dissolved with 300ml of distilled water and methanol respectively to create stock solution.

3.2. High Performance Liquid Performance Chromatography

Methodology

HPLC Procedure

3.2.1. Definition

HPLC is a method used to separate mixture of complex samples. HPLC is an active process in which materials are pumped at high pressure through a separating column, which contains a stationary phase usually a chemically functional beads that separates the compound mixtures. samples are introduced through the injector and carried via the mobile phase across the stationary phase to effect the separations. After separation through the column, the samples are expose for a detector system that identify and qualify the individual compounds.

3.2.2. Extraction

10g of sample was measured into the amber bottle. To it was added 20mls of Acetonitrile/methanol; both were shaken vigorously for 30minutes. After the shaking, the (aqueous) end was run off while the organic solvent end was collected with 25ml standard flask made up to the mark and ready for the analysis.

3.2.3. Analysis

Standard form of analytes profile were first injected into the HPLC and this generated a chromatogram with a given peak area and peak profile. These were used to create a window in the HPLC in preparation of the test sample analysis. Then the aliquot of the extracted test sample was injected into the HPLC also, to obtain a corresponding peak area and peak profile in the chromatogram. Then the peak area of the sample is compared with that of the standard, relative to the concentration of the sample.

3.2.4. CALCULATION

Concentration of sample=

Peak area of sample \times std concentration/peak area of the std.

3.2.5. HPLC Programming

Make----- Shimadzu(Nexera mx)

Column-----Ubondapak C18

Length.....100mm

ID.....4.6mm

Thickness...7um

Mobile phase-----Acetonitrile/water (methanol/water)

Flow rate-----0.08ml/min(W) (1ml/min)

5ml/min(A)

Sample injection --5ul (10ul)

Detector-----UV (254nm) (Diode array detector DAD)

Pump pressure ---15mpa

CHAPTER FOUR

RESULTS

4.0. Result

High Performance Liquid Chromatography of Dicloromethane (DCM) Fraction of *Uraria picta*

Description: HPLC

Column: RESTEK 15 METER MXT-1

Carrier: Methanol AT 5PSI

Data File: Phytochemical

Sample: DCM UP

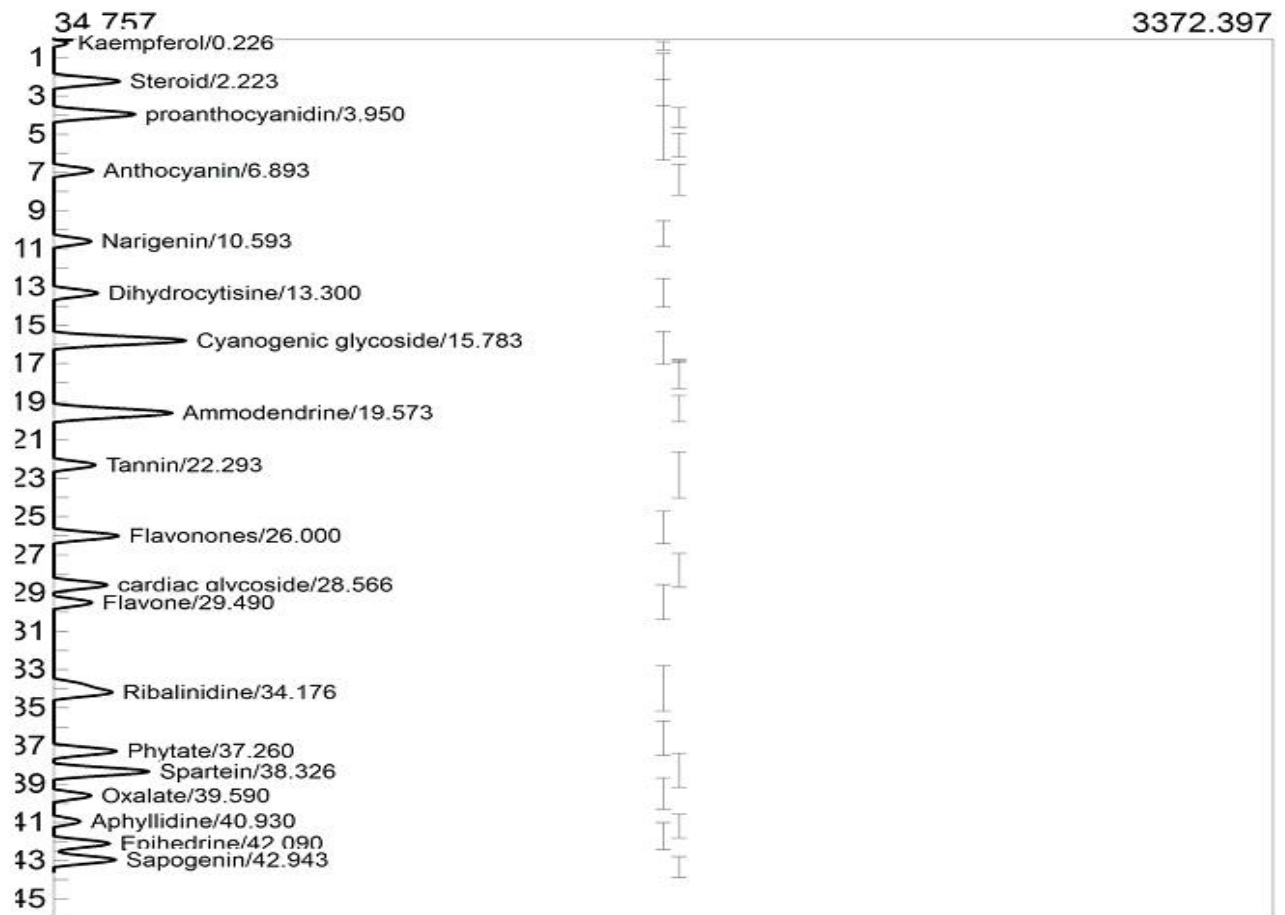


Table 4.0. Phytoconstituents from HPLC analysis of the Dichloromethane fraction of *Uraria picta*

COMPONENT	RETENTION TIME	EXTERNAL	UNITS
Kaempferol	0.226	3.3343	ug/ml
Steroid	2.223	8.2903	ug/ml
Proanthocyanidin	3.950	10.4914	ug/ml
Anthocyanin	6.893	2.0696	ug/ml
Narigenin	10.593	7.4327	ug/ml
Dihydrocytisine	13.300	5.9167	ug/ml
Cyanogenic glycoside	15.783	20.1936	ug/ml
Ammodendrine	19.573	22.0694	ug/ml
Tannin	22.293	12.9619	ug/ml
Flavonones	26.000	8.7424	ug/ml
Cardiac glycoside	28.566	4.0095	ug/ml
Flavone	29.490	5.7253	ug/ml
Ribalinidine	34.176	0.0000	ug/ml
Phytate	37.260	8.8109	ug/ml
Sparteine	38.326	15.9760	ug/ml
Oxalate	39.590	1.3133	ug/ml
Aphyllidine	40.930	4.2430	ug/ml
Epihedrine	42.090	3.9473	ug/ml
Sapogenin	42.943	10.9960	ug/ml
	121897.3705	156.5237	

CHAPTER FIVE

5.1 DISCUSSION AND CONCLUSION

The phytochemical analysis of the Dichloromethane fraction of *Uraria picta* using High performance liquid chromatography (HPLC) method, nineteen (19) compounds were identified and quantified. However, the prominent compounds include Ammodendrine (22.0694 ug/ml), Cyanogenic glycoside (20.1936ug/ml), Spartein (15.9760ug/ml), Sapogenin (10.9660ug/ml), Proanthocyanidin (10.4914ug/ml), Tannin (12.9619ug/ml). Of all the six prominent compounds quantified, Ammodendrine is the highest compound with a concentration of (22.0694 ug/ml)." The compounds that were of lower concentration include the following; steroid (8.29303 ug/ml), Narigenin (7.4327 ug/ml), Flavonones (8.7424 ug/ml), Phytate (8.8109ug/ml) Dihydrocystisine (5.9167 ug/ml), Flavones (5.97253 ug/ml), Aphyllidine (4.2430 ug/ml), Cardiac glycoside (4.0095 ug/ml), Epihedrine (3.9473 ug/ml), Kaempferol (3.3343 ug/ml), Anthocyanin (2.00696 ug/mL). Among these compounds oxalate was present in lowest concentration (1.3133 ug/ml). From the results of HPLC analysis revealed the presence of Ribalinidine in Dichloromethane fraction of leaf extract of *Uraria picta*, but its concentration in ug/ml was (0.0000 ug/ml). This indicates that the concentration of Ribalinidine in ug/ml of Dichloromethane fraction of *Uraria picta* is too small to be quantified by HPLC analysis. The identification of bioactive compounds carried out on *Uraria picta* using high performance liquid chromatography (HPLC) showed the presence of various components such as tannin, Ammodendrine, Steroid, Anthocyanin, Oxalate as the major metabolites present in the plant's leaf as shown in table 4.0 many of the secondary metabolites produced by plants are used by pharmaceutical industries (since these bioactive compounds trigger a pharmacological or toxicological effect in humans and animals), in cosmetics, nutrition, for the manufacture of drugs, dyes, fragrances, flavors, dietary supplements.

Hence, both the scientific and industrial interest around plant secondary metabolites are enormous. Kaempferol is a flavonoid, its pharmacological benefits include; Antioxidant: Kaempferol help neutralize free radicals in the body, which can reduce oxidative stress and inflammation. Anti-inflammatory: Its anti-inflammatory effects is due to the ability to inhibits inflammatory mediators such as cytokines and leukotrienes. Being a natural flavonoid, kaempferol can be administered alone or with other drugs (Wang *et al.*, 2019). proanthocyanidins may protect the heart and cardiovascular system. They may work as antioxidants and block nitrosamines from forming. They may protect healthy cells from their effects. They work with vitamin C to lower the risk of breast cancer. Tannins are natural antioxidants linked with the prevention of degenerative diseases such as atherosclerosis, cardiovascular diseases, neuro-degenerative diseases, and certain types of cancers by acting as antioxidants and antibacterial. Ammodendrine has neuroprotective properties, which can help protect the brain and nervous system from damage, Ammodendrine has anti-inflammatory properties, which can help reduce inflammation and swelling in the body. It also used as Antioxidant properties, which can help protect the body from free radicals and oxidative stress. Spartein used to treat various conditions such as cardiac arrhythmias, hypertension, and asthma (Keita Teranishi, 2020). Used as a stimulant, diuretic, and emmenagogue, Used as an oxytocic to induce uterine contractions during childbirth. - Cyanogenic glycosides have neuroprotective properties, which can help protect the brain and nervous system from damage. Cyanogenic glycosides have been reported to have anticholinergic, anti-inflammatory, antimicrobial, and antiviral effects. Narigenin belongs to the flavanone group of polyphenols. It has been shown to mitigate the severity of neurogenerative diseases such as Parkinson's disease, Alzheimer's disease and dementia due to its antioxidant effects (Goyal *et al.*, 2024). Tannins are phenolic compounds of medicinally significant due to their astringent properties. They promote rapid

healing and the formation of new tissues on wounds and inflamed mucosa. Tannins are used in the treatment of varicose ulcers, haemorrhoids, minor burns, frostbite, as well as inflammation of gums. Tannins also have antioxidant properties and are known for their ability to bind to proteins (Tuyen *et al.*, 2017). Tannins are phenolic compounds of medicinal significance due to their astringent properties. They promote rapid healing and the formation of new tissues on wounds and inflamed mucosa. Tannins are used in the treatment of varicose ulcers, haemorrhoids, minor burns, frostbite, as well as inflammation of gums. Tannins also have antioxidant properties and are known for their ability to bind to proteins (Tuyen *et al.*, 2017). Ribalinidine is a quinoline alkaloid compound with free radical scavenging activity (Nwiloh *et al.*, 2016). Dihydrocytisine is a natural alkaloid found in plants. It's structurally similar to cytosine and nicotine, acting as a partial agonist at nicotinic acetylcholine receptors. Some studies suggest that dihydrocytisine can be used in smoking cessation due to its similarity to nicotine and cytosine (Pozzi *et al.*, 2024). Phytate is a complex form of both phosphate and inositol. Its consumption can provide protection against a variety of cancers via its antioxidant properties. Due to its ability to bind to minerals, it is used as a chelating agent in various industrial processes, such as metal extraction and wastewater treatment.

CONCLUSION

The phytochemical analysis of dichloromethane fraction of *Uraria picta* leaf extracts confirms the presence of important bioactive compounds and thus possesses valuable pharmacological health benefits. Kaempferol (Flavonoid) is responsible for its antioxidant activity. However, the molecular mechanism in this study is still unclear to demonstrate its antioxidant effects, thus further research is needed to understand mechanistic insights of the plant phytochemicals to represent antioxidant properties.

APPENDIX

HPLC Programming

Make----- Shimadzu(Nexera mx)

Column-----Ubondapak C18

Length.....100mm

ID.....4.6mm

Thickness...7um

Mobile phase-----Acetonitrile/water (methanol/water)

Flow rate-----0.08ml/mind(W) (1ml/min)

5ml/mind(A)

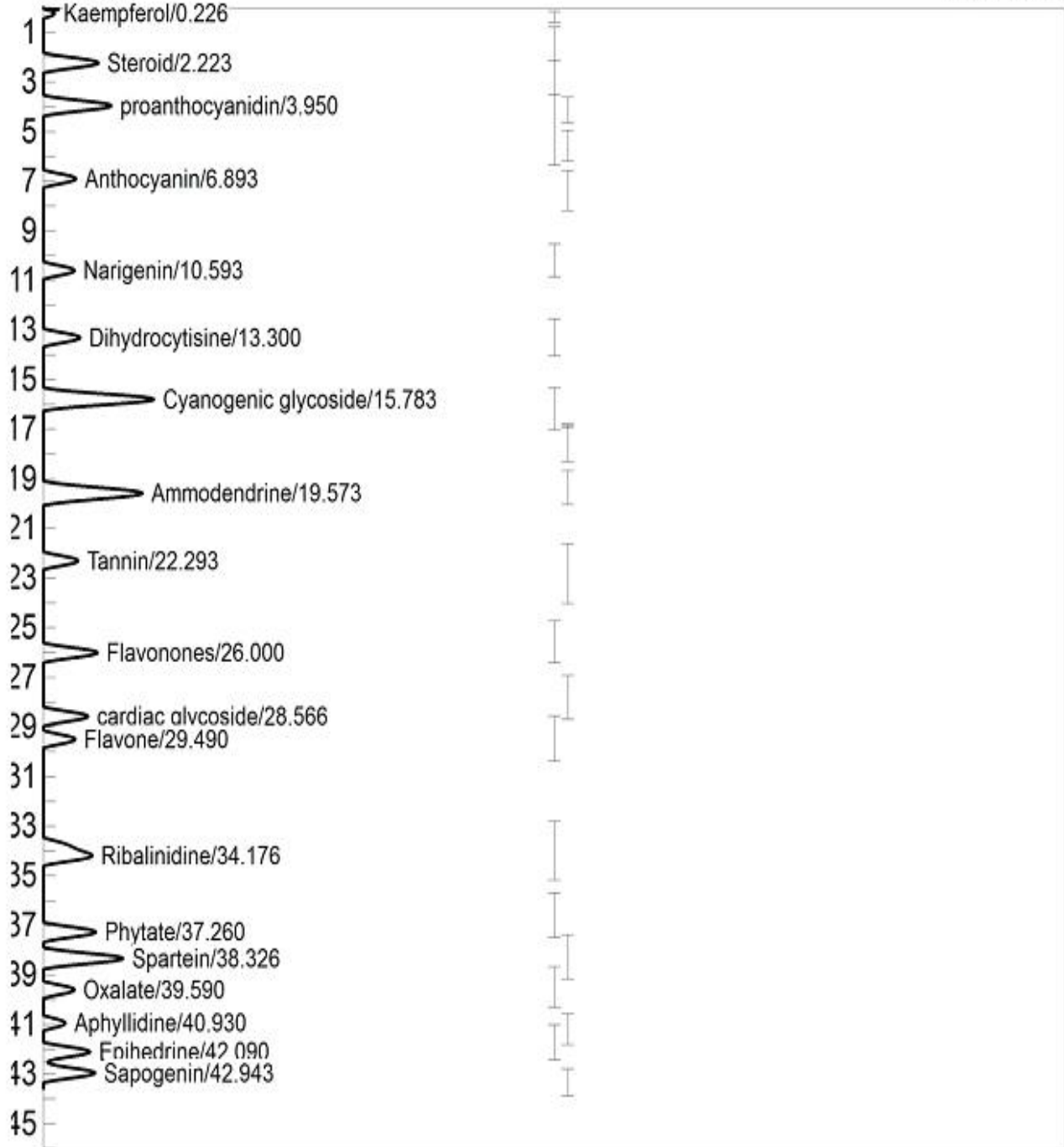
Sample injection --5ul (10ul)

Detector-----UV (254nm) (Diode array detector DAD)

Pump pressure ---15mpa

34 757

3372.397



REFERENCES

- Balasundram, N., Sundiam, K and Samman S. (2006). Phenolic compounds in plants and agricultural by product: Antioxidant activity, occurrence and potential uses. *Food Chemistry*. **126**: 1821-1835.
- Boots, A.W., Haenen, G.R., and Bast A. (2008). Health effects of quercetin from antioxidant to nutraceutical. *European Journal of Pharmacology* **585**: 325 – 337
- Cosa P., Vlietinck A.J., Berghe D.V., and Maes L., (2006). Anti infective Potential of Natural Products: How to develop a stronger invitro ‘Proof – of – concept’ *Journal of Ethnopharmacology* **106**: 209 – 302.
- Croteau, R., Kutchan T.M and Lewis N.G.(2001). Natural products (Secondary metabolite) *Biochemistry and Molecular Biology of Plant. American Society of Plant physiologists* (1250 – 1318).
- Degrou R., Kutchan T.M., Lewis N.G. (2013). Physicochemical parameters that influence Carotenoids bioaccessibility from a tomato juice. *Food Chemistry*. **(76)**: 40 – 51.
- Doughari, J.H., (2012). Phytochemicals: Extraction methods, Basic structures and mode of action as potential Chemotherapeutic agents, phytochemicals. *A Global Perspective of their Role in Nutrition and Health*. 132 – 134.
- Duraipandiyan J., Ayyanar M. and Gnacimuth S., (2006). Antimicrobial activity of some ethno medicinal plants used by Paliyar rise from Tamil Nadu, India. *Complementary Alternative Medicine*: **6**: 33 – 41.

- Duthie S.J., Ma, A.R., and Collins A.R.(1996). Antioxidant Supplementation of decreases Oxidative DNA Damage in Human Lymphocyte. *Cancer Resource*: 129 – 1295
- Goparaju, G., and Kaushal G. (2014) Significance of Stability indicating L.C. methods in pharmaceuticals. *Austin Chromatography* **18**: 1 - 2.
- Goyal A. and Iuteolin (2024). Promising waivior against Alzeimer’s and Pakison’s disease. *Biochemistry Molecular Toxicology*, 102 – 114
- Groom and Saxena H.O., Soni, A., Mohammudi, N. and Choubey S.K. (2014). *Uraria picta* in IUCN of International Union for Conservation of Nature and Natural Resources and phytochemical Screening and elemental analysis of different plant parts of *Uraria picta*. Desv A deshumul Species. *Journal of Chemistry Pharmacy Resource*. **16**: 756 – 760.
- Igboechi A.C., Osazuwa I.O. and Igwe V.E. (1989). Laboratory evaluation of acaricidal properties of extracts form *Uraria picta* (legumrnosae). *Journal of Ethnopharmacology* **26**: 293- 298
- Ingle, K.P. Deshmukh A.G, Padole D.A., Dudhare M.S., Mohari M.P. and Khekurkari V.C. (2017). Phytochemicals Extraction methods, identification and detection of bioactive compounds form plants extracts. *Journal of Pharmocology Phytochemical*. **6**: 32 – 36.
- Jakubuioski, W., and Bartosz G. (1997). Estimation of oxidative stress in Saccharomyas Cerevisae with fluorescent probes. *International Journal of Biochemistry Cell Biology*. **97**: 56 – 73
- Khare C.P. (2007). Indian Medicinal Plants: An Illustrated Dictionary Berkin Springer. 699-700.

- Khoo H.E., Azian, A., Tang S.T. and Lim S.M (2017). Anthocyanidins and anthocyanins coloured pigments as food, pharmaceutical ingredients and the potential health benefits. *Food Nutrition Resources* **13**:61 – 67.
- Maczk M., and Shahidi F. (2006). Phenols in Cereals, Fruits and Vegetables: Occurrence, extraction and analysis. *Journal of Pharmacy Biomedical Analysis* **41**: 1523-1542
- Maritin, M. and Guiochon G (2005). Effect of high liquid chromatography. *Journal of Chromatography*. **1090**: 16 – 38.
- Mohan, B., Saxena H.O., Kakkar A. and Mishra M.K., (2019). Determination of antioxidant activity total phenolic and flavonoid contents in leaves, stem and root of *Uraria picta* Desu Environmental Conservation Journal. **20**: 1- 8.
- Nwiloh, B.L., Uwakwe A.A. and Akaniwor, J.O. (2016). Phyto Chemical Screening and GC-FID analysis of ethanolic extract of root, root bark of *Sakciantida* L.Benth, *Journal of medicinal plants study*. **4(6)** 283 – 287.
- Kale, R.H., Haide, U.K. and Biyani, K.R. (2012). Protective effect of aqueous extract of *Uraria picta* in acetaminophen induced nephrotoxicity in rats. *International Journal of Resource Pharmacy Biomedical Sciences*. **3**: 110-113.
- Kammerer, D., Carle R. and Schieber A. (2004) quantification of anthocyanins in black Carrot extracts (*Daucus rota* seed) sativus var. altroben Alef) and evaluation of their colour properties. *European Food Research and Technology*. **219**: 479-486.
- Khoddami, A., Wikes M.A. and Robert, T.H. (2013). Techniques of analysis of Plant Phenolic Compounds. *Molecules*. **18**: 2328-2375

- Occhiutor and limardi (1994). Compara effects of flavonoids Luteolin, apinn and rhoifolin on experimental pulmonary hypertension in dog. *Phyto therapeutic Resource*. **4**: 153 – 1536.
- Olufemei, A.O., Omoniyi, Y.K. and Ibitayo, A.M., (2016). Mechanistic evaluation of toxic anotinoceptive, anti-inflammatory and antiantioxidant actions of heat extract of *Uraria picta* LASU *Journal of Medical Science*. **1**: 1 – 10.
- Patel, B.D., kamariya, P.H., and Patel, M.B. (2011). Antioxidant Potential of aqueous extract of entire plant of *Uraria picta*. *Desv Journal of International Pharmacy Resources* **3**: 92-96.
- Ruhman, M.M., Gibbons, S and Gray A.I. (2007). Isoflavones from *Uraria picta* and their antimicrobial activity. *Phytochemistry*. **68**: 1692 – 1697.
- Sasidnaran S., Chen Y., Saravanan D., Sundram K.M. and Yoga Latha L. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from plants extract. *African Journal of Complement Alternating Medicine*. **8**: 1 – 10.
- Saxema, H.O., Mohan B. and Kakkar A. (2006). Assessment of variation in Rhoifolin content in aerial part of *Uraria picta*. Different locations of Madhya Pradesh. *Journal of Pharmacy Resource*. **10**: 185 – 195.
- Singh, V., (2017). Medicinal plants and bime healing. *Journal of maxillofac surg*, 4-11. Nail, K. and Krishnamurthy R (2018). Antiflamatory activity of methanolic extract of Pseudarthria viscudia and *Uraria picta* against carrageenan induced paw edema in albino rat. *Journal of Medicinal Aromat Plant* **9**: 112-115.
- Subsamanian, S. and Govindasamu, S. (1995). Biochemical Studies on the antiulcerogenic activity of Cavvery 100, an ayurvedic formulation in experimental ulcers. *Indian Journal of Pharmacology*. **27**: 101 – 103.

- Suffredini I.B., Sader, H.S., Goncalus A.G., Reis A.O., Gales A.C., Uarella, A.D. and Younes R.N. (2004). Screening of antibacterial extracts from plants native to the Brazilian amazon rain forest and Atlantic forest. *Journal of Medical Biology Resource* **37**: 379 – 384.
- Tuyen P.T., Xuan, T.D. Khang D.T., Ahmad, A., Quan N.V. and Anh (2017). Phenolic composition and antioxidant properties in bark, flower, inner skin, kernel and leaf extract of
- Valko, M., Rhode C.J., Moncol J., Izakakovic, M. and Mazur, M. (2006). Free radicals metals and antioxidants in oxidative stress-induced cancer. *Chemical Biology of Interaction* **160**: 2005 – 2009
- Vivekanthan, D.P., Penn, M.S., Sapp, S.K., Hsu, A. and Topol, E.J. (2003). Use of Antioxidant Vitamins for the Prevention of Cardiovascular Disease: Meta-analysis of Randomized Trials. *Lancet* **361**: 2017 – 2023.
- Wang Y., Liv H., Lev, C., Deng J., Yan Y., Chen H., Liang C.I, Wei J., Han L. and Dai Z., (2019). Kaempferol attenuates imiquimod induced psoriatic skin inflammation in a mouse-model. *Clinical and Experimental Immunology*. **3**: 403 – 415.
- Ye, M., Han, J., Chen H., Zhen J and Guo D. (2007). Analysis of phenolic compounds in rhubarbs using liquid chromatography occupied coupled with electrospray ionization mass spectrometry. *Journal of Mass Spectrometry*. **18**: 82 – 91.
- Zotchev S.B. (2013). Chapter eleven-alkaloids from marine bacterial in Giglide-Guivarch N(ed) *Advances in Botanical Research*. 122-133.