Comparative Phytochemical and Analgesic Evaluation of Methanolic Extract of *Tecoma stans* Leaves

Thesis submitted in partial fulfillment of the requirements for the Degree of Master of Pharmacy

Submitted by

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EXAM ROLL NO.: M4PHL24011

CLASS ROLL NO.: 002211402037

REG. NO.: 163679 of 2022-23

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Session- 2022-2024

August 2024

CERTIFICATE OF APPROVAL

This is to certify that the thesis entitled **"Comparative Phytochemical and Analgesic Evaluation of Methanolic Extract of *Tecoma stans* Leaves"** submitted by **Dipanwita Pramanik**, Examination Roll No.: **M4PHL24011**, Registration No.: **163679 of 2022-23**, for the partial fulfilment of degree of **Masters of Pharmacy**, Jadavpur University, is absolutely based upon her own research project work under my supervision, in the Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.

She has included her finding into this thesis. Her thesis has not been submitted before for any degree/diploma or any other academic award elsewhere. I am satisfied that she carried out her thesis with proper care and confidence to my satisfaction.

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Acknowledgement

The final outcome of this dissertation required a lot of guidance and assistance from many people. I am extremely fortunate to have these all along the completion of my work. Whatever I have worked only due to such guidance and assistance and I would not forget to thank them. I am highly obliged and like to express my deep gratitude and profoundness to my reverend mentor **Dr. Emdad Hossain** of Department of Pharmaceutical Technology, Jadavpur University, Kolkata for his excellent and constant guidance and help, endless encouragement, thoughtful and freedom and stupendous co-operation throughout the dissertation till its successful completion and also thankful to **Prof. (Dr.) Subhash C. Mandal** for his guidance throughout my project. I am also grateful to **Dr. Nilanjan Ghosh** for his support in research work.

I am greatly indebted to his motivation, fruitful suggestions and inspirations. I owe my deep respect to **Prof. (Dr.) Amalesh Samanta**, Head of the Department, Pharmaceutical Technology, Jadavpur University, Kolkata for all the necessary help and encouragement. I am thankful to all of my respected teachers and non-teaching staffs in the Department of Pharmaceutical Technology, Jadavpur University for their support and help. I am both extremely honoured and grateful to Research Scholars Mr.Shuvam Sar, Mr. Tanmoy Banerjee, Mr. Akash De for their priceless guidance and support which assisted me to gather knowledge about the different aspects of this work. I also express my sincere thanks to my classmate Mr. Tathagata Khanra, laboratory colleague Mr. Sandeep Jana, Mr. Tuhin Baran Samoi, and laboratory juniors Mr. Tapendu Naskar, Ms. Karma Lama of Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Technology Jadavpur University, Kolkata.

I would like to convey my sincere gratitude to AICTE and Jadavpur University for their financial and equipmental support for my M. Pharm course. Finally, I would like to express my deep respect to my Father Late Subhankar Pramanik, my Mother Madhuri Pramanik, my elder sister Tanuva Dey and also my other friends and relatives for their continuous help, love, encouragement and moral support throughout the period of my work.

Dipanwita Pramanik
29.08.24

(Dipanwita Pramanik)

DECLARATION

Declaration of Originality and Compliance of Academic Ethics I hereby declare that this thesis contains literature survey and original research work performed by me (Dipanwita Pramanik) as a part of my Master of Pharmacy studies. All the information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have cited and referenced the materials and results that are not original to this work. Name: **Dipanwita Pramanik** Exam Roll Number: M4PHL24011 Class Roll Number: 02211402037 Registration Number:163679 of 2022-23, Thesis Title: "Comparative Analgesic Evaluation of Methanolic Extract of *Tecoma stans* Leaves" Dedicated to My father, mother, Elder Sister, seniors, friends and teachers.

Dipanwita Pramanik
29.08.24
(Dipanwita Pramanik)

TABLE OF CONTENT

1. Introduction:

Herbal remedies are natural substances derived from plants that are used to treat ailments within the customs and practices of local or regional medicinal traditions. These preparations consist of complex blends of organic compounds sourced from various parts of a plant either in their raw form or processed. The origins of herbal medicine can be traced back to ancient civilizations across the globe. While each culture may have its own unique approach, all traditional systems agree on adopting a holistic mindset towards improvement of health. Prominent examples include Traditional Chinese Medicine and Ayurvedic Medicine in India which promote the concept of prioritizing overall wellness rather than solely targeting specific illnesses. The use of healing plants allows individuals to thrive and focus on improving their overall state as opposed to addressing a specific ailment that is often a result of imbalances in the mind body and surroundings. Herbal medicine has been practiced since ancient times utilizing medicinal plants to alleviate disease symptoms and promote general health and wellbeing. Also known as herbalism or botanical medicine this therapeutic system is based on the utilization of plants or their extracts which can be ingested or applied topically. Since primitive ages various cultures worldwide have utilized including herbal remedies to address a wide range of conditions such as malaria, warts, digestive issues, cardiovascular disorders and chronic pain. Much of this knowledge has been passed down through generations and has been integrated into mainstream medicine by pharmacists and doctors. However there have also been instances where members of the community have been reluctantly tasked with passing on traditional remedies often without compensation. Ronnie Moore a sociology professor explained that these individuals may not accept payment for their services viewing it more as a burden than a gift. Nonetheless they feel a strong sense of obligation and reverence towards the duty of healing a deep-rooted part of life and death. It may surprise to some people but approximately 25% of all prescribed medications worldwide are derived from plants. Moreover, the World Health Organization's listed essential medicines which includes around 11% that are exclusively from plant-based. Over 200 years ago, the first pharmacological compound morphine was extracted from the opium found in the seed pods of poppy flowers. Scientists have delved into the study of plants to formulate many of the pharmaceutical products available today. However, with the growing concern surrounding overmedication, antibiotic-resistant bacteria in

the body and the treatment of symptoms rather than the root cause, more attention is being directed towards the use of natural herbal medicine (Tapsell *et al*.,2006).

A vast sum of money has been recently devoted to the pursuit of potential curative plants. These significant expenditures on exploring traditional botanical remedies while relatively modest compared to the booming pharmaceutical industry demonstrate a shift towards unconventional drug development and a greater focus on natural healing methods. Throughout human history natural plant products have served a variety of purposes. Dating back over 5000 years written accounts of the utilization of herbal medicine attest to its longstanding role as the sole form of treatment. Presently plants are being harnessed to address an array of health issues ranging from allergies, arthritis to migraines, fatigue skin disorders, wounds, burns, gastrointestinal ailments and even cancer - a testament to the adage that 'food is medicine'. These herbs are more affordable and offer a safer alternative to conventional drugs fuelling an increasing number of individuals returning to these traditional healing practices. While botanical medicine has endured for thousands of years it remains relevant in the Modern Western World. A recent estimate by the World Health Organization revealed that 80% of people globally rely on herbal remedies for at least some of their primary healthcare needs with the market for such products reaching nearly $60 billion annually worldwide. In the United States interest in herbal medicine has surged due to the escalating cost of prescription drugs and a renewed enthusiasm for natural and organic remedies (Lai, P.K. and Roy, J., 2004).

2. Plant Introduction:

Tecoma stans (L.) Juss. ex Kunth also known as Kusi urakame, Koyawari, Palo amarillo and Tronadora (Irigoten-Rascon and Paredes, 2015) belongs to the Bignoniaceae family and is commonly referred to as yellow-elder, yellow trumpet bush, trumpet-flower, yellow-bells trumpet, bush ginger-thomas esperanza timboco. This plant can be found in the mountainous regions of South America as well as in dry areas of North America. It has successfully adapted to tropical and subtropical climates and can now also be found in Africa, Asia and Oceanica (https://www.cabi.org). Due to its beautiful clusters of bright yellow cup-shaped flowers evergreen leaves and bountiful fruits and seeds *Tecoma stans* is often grown as an ornamental plant (White 2003). The medicinal potential of this plant has been recognized and it has been listed in the Plant List and the Medicinal Plant Names Services (https://www.theplantlist.org; https://mpns.science.kew.org). As far back as 1570 royal physician Hernandez first described the uses of *Tecoma stans* in his work. Over time Mexican scientists have further studied that the plant has confirmed its efficacy in treating hyperglycemia through leaf infusion. Natively various parts of the plant have been used for treating a range of conditions such as headaches, intestinal disorders, jaundice, kidney problems, skin infections, toothaches, joint pains, sore eyes and heart pain. Furthermore, it is commonly used as an antidote for snake, scorpion and rat bites. Numerous pharmacological studies have described the medicinal properties of *Tecoma stans* including its hypoglycemic, hypolipidemic, anti-cancer, antioxidant, immunomodulatory, analgesic, anti-microbial, anti-spasmodic, wound healing and hepatoprotective (Anburaj *et al*., 2016a,b,c; Bakr *et al*., 2019; Pullaiah, 2002; Pullaiah and Naidu, 2003; Prajapati and Patel, 2010; Rajamurugan *et al*., 2013; Verma, 2016). To this point approximately 120 compounds have been discovered and separated from the plant. Some of the vital elements include monoterpene alkaloids, carotenoids, phenolic acids, flavonoids, terpenoids, glycosides, phytosterols, volatile oils and unsaturated fats (Taha 1954; Sbihi *et al*. 2015; Taher *et al*. 2016; Alade *et al*. 2019; Mohammed *et al*. 2019). Of these monoterpene alkaloids, phenolic acids, flavonoids and fatty acids are the key bioactive substances responsible for its medicinal properties. For example, tecomine and chlorogenic acid found in the plant's leaves have demonstrated the ability to lower glucose levels (Rodriguez de Sotillo and Hadley 2002; Constantino *et al*. 2003a, b). In addition, researchers have explored the potential use of apigenin and chrysoeriol extracted from the leaves as main components of a drug

to inhibit pancreatic lipase enzymes (Ramirez *et al*. 2012). Apart from its medicinal value the plant is also utilized as a lubricant cosmetic flavoring agent and in perfumery (Dr. Dukes Phytochemical and Ethnobotanical Database Agricultural Research Services USDA). More recently scientists have successfully synthesized silver nanoparticles using the plant's leaves and flowers expanding its potential applications in biomedicine, food packaging and wound healing (Arun K. *et al*. 2013).

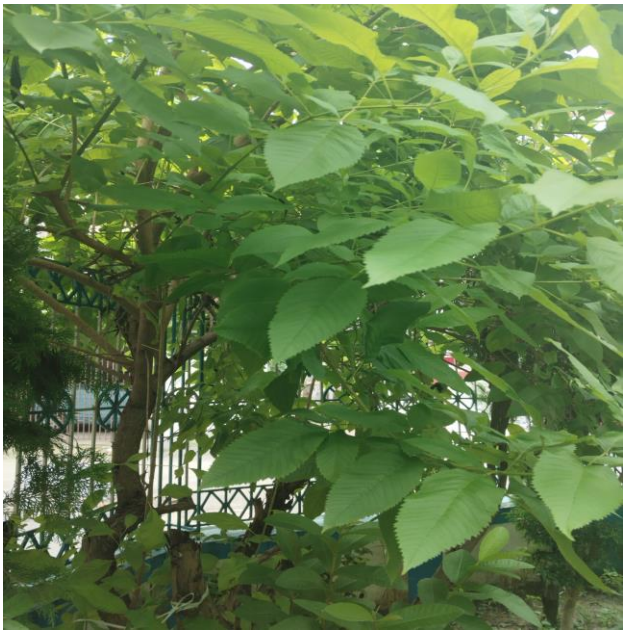


Figure: 1a

Figure: 1b

Figure: 1c

Figure: 1d

3. Morphological description

This is a medium-sized evergreen flowering perennial shrub. It sheds its leaves in the falling period. The plant grows at a height of 1.5 to 7.5 meters and has a width of 1 to 2.6 meters (Figure 1a). Its lower stems have a smooth bark which turns a light shade of grey as it matures. When young the bark is adorned with small scales and as it grows deep grooves and furrows form. The leaves of this plant are green compound and have serrated edges (5-13 leaflets). These leaflets are arranged alternately along the wingless midrib with the exception of one terminal leaflet. The underside of the leaves contains a slight fuzz near the midrib and in the vein axils. Each leaf measures 5-8 cm in length and 4 cm in width and has a short stalk. They are either lanceolate elliptic or ovate in shape and have a sharp tip with a narrow base (Figure 1b). The flowers of this plant are of bright yellow color. They grow to about 4-5 cm in length and have a short stalk. The tubular shape of the flowers is accentuated by faint orange to red stripes running down its throat. These flowers are arranged in clusters on long racemes with each cluster containing up to 50 blooms. As the branches bend under the weight of the flowers, they form elegant arches and resemble golden bells from afar (Figure 1c). In India the plant bursts into bloom during the fall season with a shorter flowering period in spring. The alluring scent of these flowers attracts various pollinators such as bees, hummingbird's, butterflies and more. The fruit of this plant measures 10-25 cm in length and takes on a bean-like shape. It is made up of two sections each of which can hold around 10-20 seeds (Figure 1d). At early stage the fruit is green but turns a pale shade of brown as it matures. The dried fruits stay on the tree for several months forming untidy clusters. Eventually they split open releasing numerous papery and winged seeds which float away to nearby areas with the breeze (Verma, 2011; Dhanya *et al*., 2013; Labhane and Dongarwar, 2014).

4. Geographical distribution, habitat, and propagation

4.1. Geographical distribution

The indigenous range of this particular plant spans from the southern regions of Texas, New Mexico and Arizona to Bolivia and Northern Argentina and from Florida. It has also spread naturally to various parts of tropical and subtropical countries such as Africa (including Botswana Cabo Verde, Ethiopia, Kenya, Malawi, Mauritania, Mauritius, Nigeria, Rwanda South Africa Tanzania Uganda Zambia and Zimbabwe) Asia (specifically India and Indonesia) and Oceania (including American Samoa, Western Australia, Queensland, Christmas Island, Cook Island, Federated States of Micronesia, Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, New Caledonia, Niue, Northern Mariana Islands, Palau, Samoa, Solomon Islands and Tonga) (FAO 1986; https://www.cabi.org). In India this plant can be found extensively from the Shiwalik ranges in the Himalayas all the way to the plains in the southernmost part of India. It has been documented in various regional floras across the nation including Shimla in Himachal Pradesh (Thakur *et al*. 2012); Nalgonda, Baptala, Tirumala hills, Puttaparthi, Simhachalam and Warangal in Andhra Pradesh (Prasanna *et al*. 2013; Anand and Basavaraju 2016); Bargarh and Cuttak in Orissa (Das *et al*. 2010; Dash *et al*. 2011; Giri *et al*. 2012); Chennai Hosur and Namakkal in Tamil Nadu (Ghandhi and Ramesh, 2010; Thirumal *et al*., 2012; Kameshwaran *et al*., 2012; Rajamurugan *et al*., 2013); Pune in Maharashtra (Torane *et al*., 2011); Modasa in Gujarat (Prajapati and Patel, 2010); Karnataka and Western Ghats (Shanmukha *et al*., 2013a,b).

4.2. Habitat

This species of plant thrives in areas with high levels of sunlight and is often found along roadsides and in disturbed environments. It cannot tolerate extreme frost or strong winds but it can withstand moderate levels of salt in the soil and is highly resilient to drought. It has the ability to grow in a variety of well-drained soils as long as they receive an average rainfall of 7001800 m. While it prefers clay loams it can adapt to most types of soil including alkaline conditions. It typically grows for 10-20 years enduring disturbance (Tipton, 1994; Lohmann, 2006; Orwa *et al*., 2009).

4.3. Propagation

The species reproduces via seeds and can also be propagated through green cuttings. The seedlings are easily transplanted and typically reach maturity within two years. During the initial stages of growth, the plant demonstrates rapid expansion and can reach heights of up to 1 m. While they are able to thrive in higher altitudes, their flowering may be stunted. Young plants require frequent watering and protection from livestock but once established they can withstand unpredictable irrigation and minimal care. Regular pruning is necessary to regulate their growth. Following a period of abundant blooming some plants may become partially deciduous and suffer from chlorosis. This makes it an opportune time to trim them as the interior of the plant is more accessible. The species may fall prey to insects, parasitic plants and disease-causing organisms yet none of these pose a significant threat (White 2003).

5. Traditional uses

The various components of the plant have been traditionally utilized in herbal medicine to address a wide range of ailments and conditions. This particular plant has been widely incorporated into Mexican traditional medicine to alleviate elevated blood sugar levels, treat intestinal liver and skin disorders and provide relief from toothaches, headaches, joint pains and common colds. Furthermore, it is commonly utilized as a potent diuretic and an effective antidote against scorpion, snake and rodent bites. The Tara Humaras, a native American tribe with significant populations residing in Mexico's Chihuahua, Durango and Sonora regions refer to this plant by various names including Kusi Urakame, Koyawari, Palo Amarillo and Tronadora. This group continues to extensively utilize Tecoma as a home remedy. The flowers of the plant are brewed into tea and consumed to alleviate symptoms of the common cold while the chest is also rubbed with it during episodes of heart pain. This population also utilizes the plant as a remedy for sore eyes (Irigoten-Rascon and Paredes 2015). Similarly, the leaf of the plant is used by the rural community of Kyaing Tong Township in Myanmar to heal fractures relieve joint pains associated with rheumatoid arthritis and treat fevers and snake bites (Moe and Hlaing 2019). In India the plant is known for its antidiabetic properties and it is also used as a therapeutic agent against snake, scorpion and rodent

bites in the Satara and Salem districts of Maharashtra and Tamil Nadu (Thangadurai 1998; Mishra *et al*. 2008).

6. Chemical composition

6.1. Nutritional components

The leaf samples exhibited a range of moisture levels spanning from 5.90% to 11.75% according to studies conducted by Prajapati and Patel, (2010); Rao *et al*., (2010); Sunamola *et al*., (2012) and Abere and Enoghama, (2015). Vargas-Figueroa and Torres-Gonzalez (2018) also found a moisture content of 7.7% in seeds. Additionally, research has shown that the callus induced from leaves contained various sugars including glucose, fructose, sucrose and xylose as documented by Dohnal in both 1976 and 1977. Furthermore, Sunamola *et al*., (2012) observed high levels of carbohydrates (46.27 g/100 g dw) and crude fiber (9.25 g/100 g dw) in leaf tissue. Protein content ranged from 20.24 to 26.24 g/100 g dry weight in leaf as reported by Sunamola *et al*., (2012) and Rodriguez *et al*., (2015). The total ash content in leaf specimens ranged from 5.02 to 13.00 g/100 g dry weight based on findings from Prajapati and Patel, (2010); Rao *et al*., (2010); Sunamola *et al*., (2012); Agarwal and Karthikeyan, (2014); Abere and Enoghama, (2015). In a study conducted by Sbihi *et al*., (2015) the fat composition of plant seeds was determined to be 15 g/100 g dry weight with a high percentage (89%) of unsaturated fatty acids. Interestingly Sunamola *et al*., (2012) reported a significantly lower crude fat content of 2.57 g/100 g dry weight in plant leaves. In another investigation by Maiti *et al*., (2016) the mineral concentration (mg/100 g dry weight) was examined and found to include potassium (5722) phosphorus (136) magnesium (31.0) iron (2.64) zinc (2.95) and copper (2.57) in leaf samples. Additionally, the carbon (48.8) and nitrogen (3.28) levels were also analyzed. Sunamola *et al*., (2012) documented the amount of calcium iron, zinc and copper to be 145.99 8.147 2.636 and 0.66 mg/100 g dry weight respectively in leaf samples.

6.2. Phytochemical profile

Upon examination the components of the plant were found to contain substances such as polyphenols, flavonoids, terpenoids, alkaloids, glycosides, phytosterols, saponins, tannins and fatty acids. From the various solvents utilized methanol and ethyl acetate yielded the highest

amount of extraction and presence of these phytochemicals (Raju *et al*. 2011; Taher *et al*. 2016). In 1959, Hammouda and Motawi made a breakthrough by identifying the first alkaloid in the plant's leaves which they named tecomine (Fig. 2d). Subsequently in 1966 Hammouda and Amer discovered another alkaloid tecostanine (Fig. 2e). Other leaf alkaloids that have been reported include boschniakine (Fig. 2a), 4-hydroxytecomanine (Fig. 2b), N-normethylskytanthine (Fig. 2c), 7-hydroskytanthine, 5-hydroskytanthine, γ-skytanthine, tecomanine and 4-noractinidine. As research progressed there was a shift towards identifying and determining other functional molecules present in the leaf as well as other plant parts. Various biologically active compounds have been identified in the leaf including phenolic compounds such as chlorogenic (Fig. 3a), cinnamic acid (Fig. 3b), ferulic acid (Fig. 3c), gallic acid (Fig. 3d) as well as caffeic acid, o-coumaric acid, vanillic acid, and sinapic acids. Other components found in the leaf are sitosterols, triterpenoids and flavonoids such as flavonone apigenin (Fig. 3e), chrysoeriol (Fig. 3f), kaempferol (Fig. 3g), luteolin (Fig. 3h), quercetin (Fig. 3i), rutin (Figs. 3j), 7 8-dihydroxy-4 6-dimethoxy flavone and verbascoside (Rastogi and Mehrotra, 1990; Lins and Felicio, 1993; Srivastava, 1994; Marzouk *et al*., 2006; Ramirez *et al*., 2016).Various active ingredients including 2-(3, 4-dihydroxy phenyl) ethyl-2-O-[6 - deoxy - α- L – mannopyranosyl 4 - (3 4 - dihydroxy phenyl) -2- propenoate]-β-D-glucopyranose, 4-O-caffeoyl-α-L-rhamnopyranosyl- (1–3)-α/β-D-glucopyranose, E/Z acteoside, isoacteoside, 5-hydroxyskytanthine hydrochloride, rutin, luteolin 7-O-β-D-neohespridoside and luteolin 7-O-β-D-glucopyranoside have been identified in the pods. The presence of these same compounds has also been observed in plant flowers such as luteolin 7-O-β-Dglucuronopyranoside, diosmetin 7-O-β-D-glucopyranoside, diosmetin luteolin 7-O-β-D-glucopyranoside, diosmetin 7-O-β-D-glucuronopyranoside methyl ester, and acteoside (Anburaj *et al*., 2016).

6.2.1. Leaves, branches, and bark

In the study conducted by Govindappa *et al*., (2011) the quantification of total phenolic compounds (mg GAE/g extract) revealed values of 216, 206 and 177 for methanolic, ethanolic and aqueous extracts respectively. However, Dash *et al*. (2011) described the presence of flavonoids, alkaloids, tannins, saponins and phenols. In a separate study carried out by Salem *et al*., (2013), it was observed that the methanolic extract of *Tecoma stans* had the highest concentration of polyphenols

and flavonoids in both leaf and branch samples compared to other extracts like ethanolic aqueous and n-butanol. Interestingly the chloroform extract did not contain any significant amounts of these phytochemicals. The determination of total phenolic content (mg GAE /g extract) in methanolic extract showed values of 37.7 and 50.3 in branches and leaves respectively whereas ethanolic extracts recorded 30.33 and 44.1 followed by 20.67 and 24.3 in n-butanol extracts and 23.66 and 25.33 in aqueous extracts of branches and leaves, subsequently.

Aarland *et al*. (2015) evaluated the methanolic leaf extract (dry weight) and identified active compounds including total phenols (411 GAE mEq/g), flavonoids (5.76 quercetin equivalent mEq/g) and chlorogenic acid (34.6 ppm). However, caffeic acid was not observed in their research. In contrast Taher *et al*. (2016) quantified the total phenolic and flavonoid content in crude methanolic, methylene chloride, ethyl acetate, butanol and crude flavonoid extracts from leaves. The ethyl acetate extract demonstrated the highest concentrations of total phenols (279.41 mg GAE/g extract) and flavonoids (59.91 mg QE/g extract) followed by the crude flavonoid (264.7 and 55.55), crude methanol (230.3 and 51.19), butanol (232 and 45.75), and methylene chloride (102.49 and 39.21) extracts, subsequently. The use of high-performance liquid chromatography (HPLC) revealed the presence of 23 total phenolic compounds (mg/100 g dw) in the methanolic extract. Rutin (112.7) dominated the composition accompanied by other derivatives such as ferulic acid (31.38), pyrogallol (29), rosmarinic acid (27.1) ,3 4 5-trimethoxy cinnamic acid (21.56), naringin (21.4) ,chlorogenic acid (17.04), quercitrin (16.6), quercetin (14), protocatechuic acid (11.01), catechin (10.74), ellagic acid (8.91), hesperetin (7.97), p-hydroxybenzoic acid (6.28), vanillic acid (5.87) ,isoferulic acid (5.02), apigenin (3.9), kaempferol (2.8), p-coumaric acid (1.49) ,caffeic acid (1.40), gallic acid (0.9), 7-hydroxy-flavone (0.9) and cinnamic acid (0.31). These findings demonstrate the diverse array of phytochemicals present in plants and the specific nutrient requirements during different growth stages. (GAE: Gallic acid equivalent, QE: quercetin equivalent, dw: dry weight).

In their study Anand and Basavaraju (2016) conducted a comparative examination on the leaves of Tecoma plant at two different stages: flowering and pre-flowering. They utilized various solvents including aqueous, methanol and ethyl acetate to qualitatively analyze the presence of phytochemicals. The results revealed that the flowering phase had higher concentrations of polyphenols, flavonoids and tannins compared to the vegetative phase with the most significant

amounts found in the aqueous, methanol and ethyl acetate extracts. Interestingly the pre-flowering stage showed slightly elevated levels of alkaloids especially in the methanol and chloroform extracts. On the other hand, saponins and terpenoids did not show any significant changes between the two physiological stages and were mainly extracted in the methanolic extract. Notably the aqueous extract did not contain any alkaloids and terpenoids during both plant stages. These findings suggest that phenolic compounds play a crucial role in the growth regulation and structure of plants as evidenced by their higher accumulation during the flowering stage (3984.2 mg/100 g dw for polyphenols 199.2 mg/100 g dw for flavonoids and 14.8 mg/100 g dw for tannins) compared to the vegetative phase (2236.8 mg/100 g dw for polyphenols 118.4 mg/100g dw for flavonoids and 11.5mg/100g dw for tannins).Alkaloids have been observed to fluctuate throughout the distinct developmental phases of a plant and generally peak during the budding period. As the seed-bearing phase approaches there is a notable rise in alkaloid levels only to decrease as the seed ripens. In their research the authors noted a significant amount of 2450 mg/100 g dw of alkaloids during the pre-flowering stage compared to 1783 mg/100 g dw during the flowering stage. This indicates that the leaves contain varying amounts of phytochemicals influenced by the plant's physiological state as well as the type of extraction method and its efficacy. Moreover, the plant has ability to withstand at harsh environmental conditions such as high temperatures, drought, intense sunlight and soil salinity can also play a role in stimulating alkaloid biosynthesis and accumulation at different stages of growth.

6.2.2. Flowers

In the year 1954 Taha successfully separated two carotenoids β-carotene and zeaxanthin from the vibrant yellow bell flowers. The overall amount of carotenoids present was 12.6 mg per 100 g of flower. In the year 2015 Ranjit and colleagues discovered a high concentration of flavonoids measuring 195 mg QE per gram of extract in the aqueous solution and 160.5 mg QE per gram in the chloroform extract of the flower.

6.2.3. Seeds

The plant seed oil contains various types of fatty acids including octadecatrans-3, cis-9 ,12, 15-tetraeonic, palmitic, stearic, octadecadienoic, octadecatrienoic and octadecatetraenoic. These were identified in studies conducted by Hopkins and Chisholm (1965); Yoganarasimhan (1996) and Khare (2008). In a more recent study by Sbihi *et al*. (2015) the fatty acid composition was analyzed using gas chromatography revealing that unsaturated fatty acids (UFAs) made up 89.43% of the total fat content. The most abundant fatty acid was linoleic acid (45.47%), followed by oleic acid (23.56%), (11.48), palmitic acid (6.09%), stearidonic acid (6.65%), stearic acid (4.12%) and γ-linolenic acid (1.04%). Visual representations of linoleic acid and oleic acid are shown in Fig. 4 (a and b). The vitamin E, polyphenol and flavonoid levels of the oil sample were thoroughly examined. On analysis using HPLC it was found that the total amount of vitamin E present was 266.06 mg/100 g of oil. The highest percentage was of γ-tocopherol (78.93%) followed by δ-tocotrienol (18.63%). Additionally, the oil contained 168.69 mg GAE/100 g of polyphenols and 5.54 mg CAE/g of flavonoids. The extracted seed oil has a high iodine value of 180.4 g/100 g indicating its high unsaturated nature. It also contains pigments like chlorophyll (1.84 mg/kg) and carotenoids (2.49 mg/kg) in the seed oil. The oil contains valuable antioxidants such as carotenoids, vitamin E, polyphenols and flavonoids which not only have positive effects on health but also help maintain the oil's stability and extend its shelf life. Furthermore, the unsaturated fatty acids found in the oil hold significance for both the food and pharmaceutical sectors and can be utilized in creating nutritional supplements for overall well-being and remedial purposes. As a result of its exceptional fatty acid composition and presence of protective antioxidants in seed oil has the potential to be utilized as a fresh source of plant-based oil.

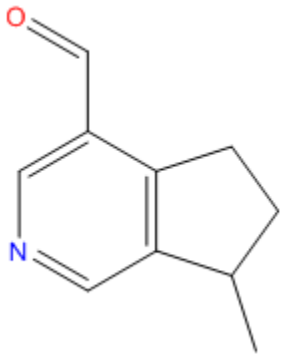


Figure 2a:-Boschniakine

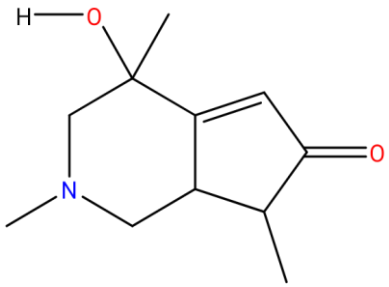


Figure 2b:- 4-hydroxytecomine

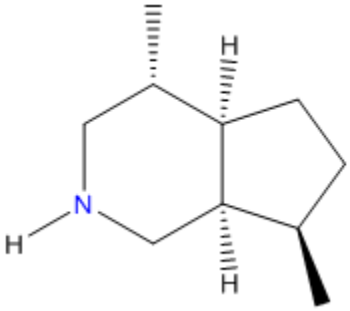


Figure 2c:- N-normethylskytanthine

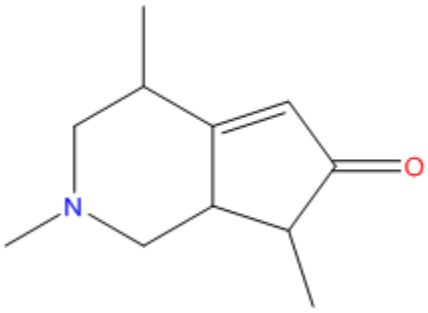


Figure 2d:- Tecomine

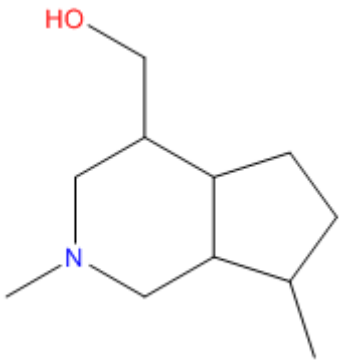


Figure 2e:- Tecostanine

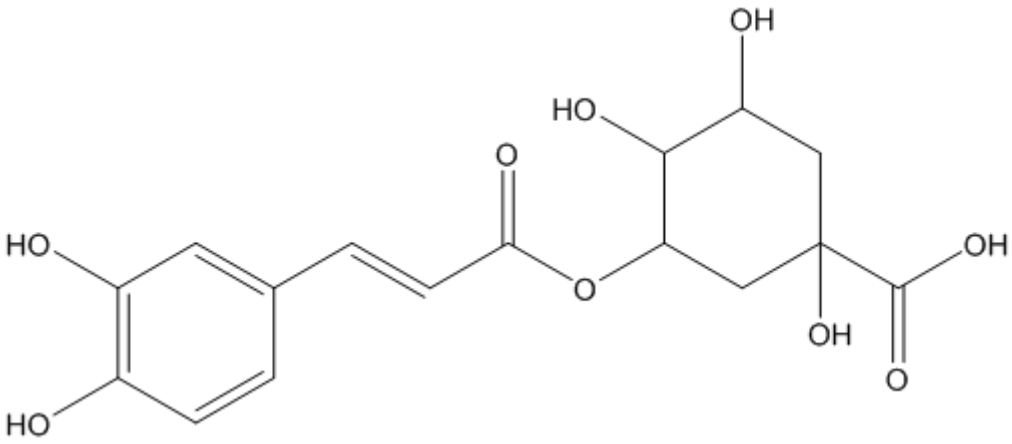


Figure 3a: Chlorogenic acid

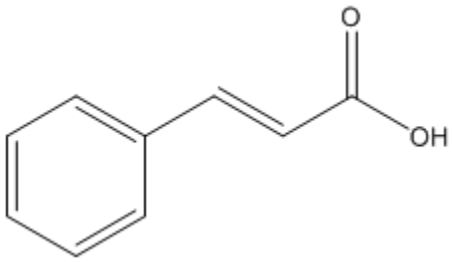


Figure 3b:- Cinnamic acid

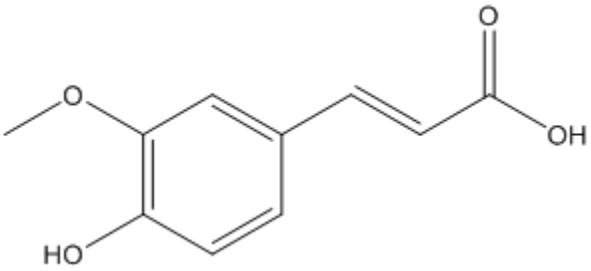


Figure 3c:- Ferulic acid

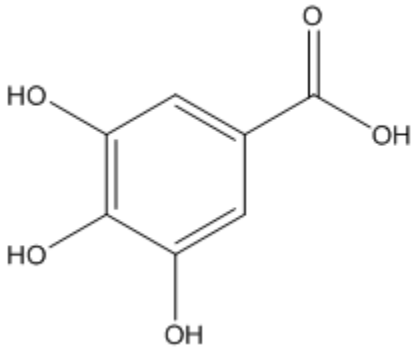


Figure 3d:- Gallic acid

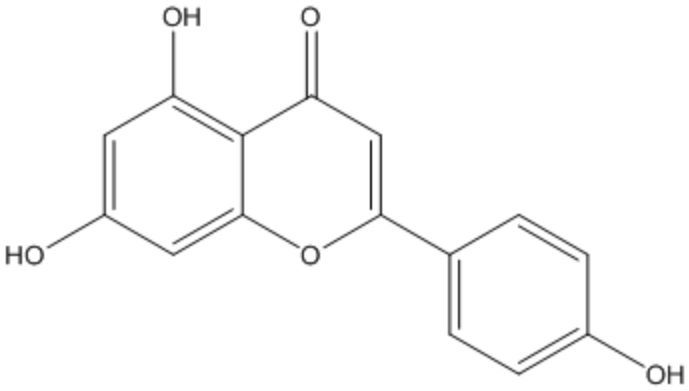


Figure 3e:- Apigenin

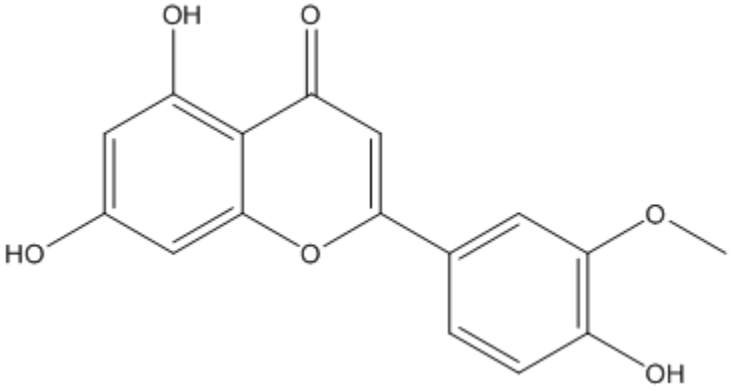


Figure 3f:- Chryseriol

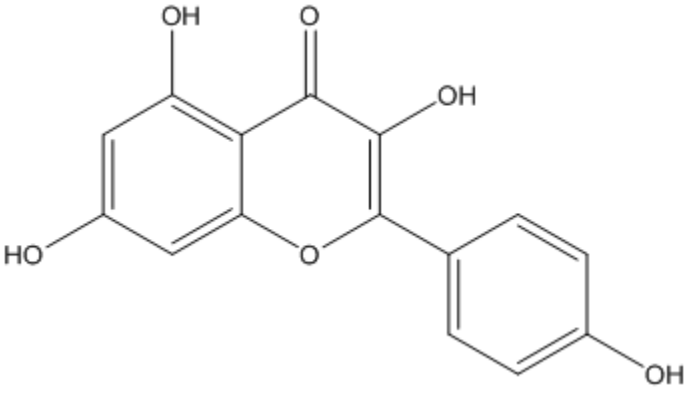


Figure 3g:- Kaempferol

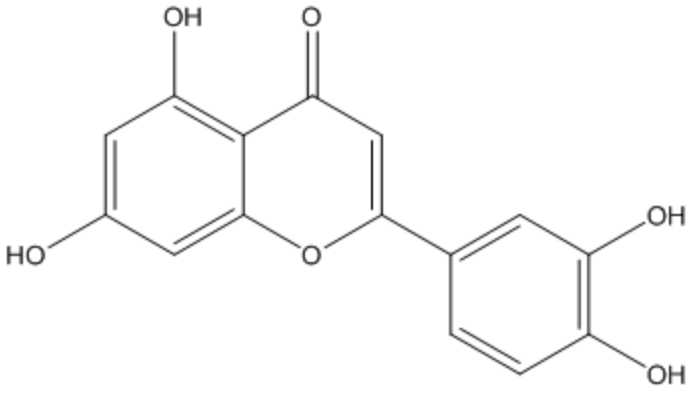


Figure 3h:- Luteolin

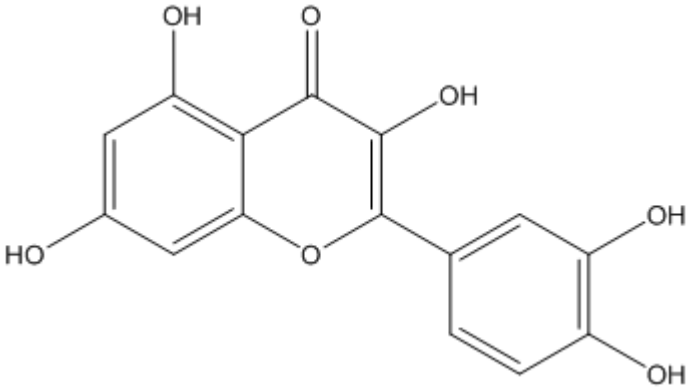


Figure 3i:- Quercetin

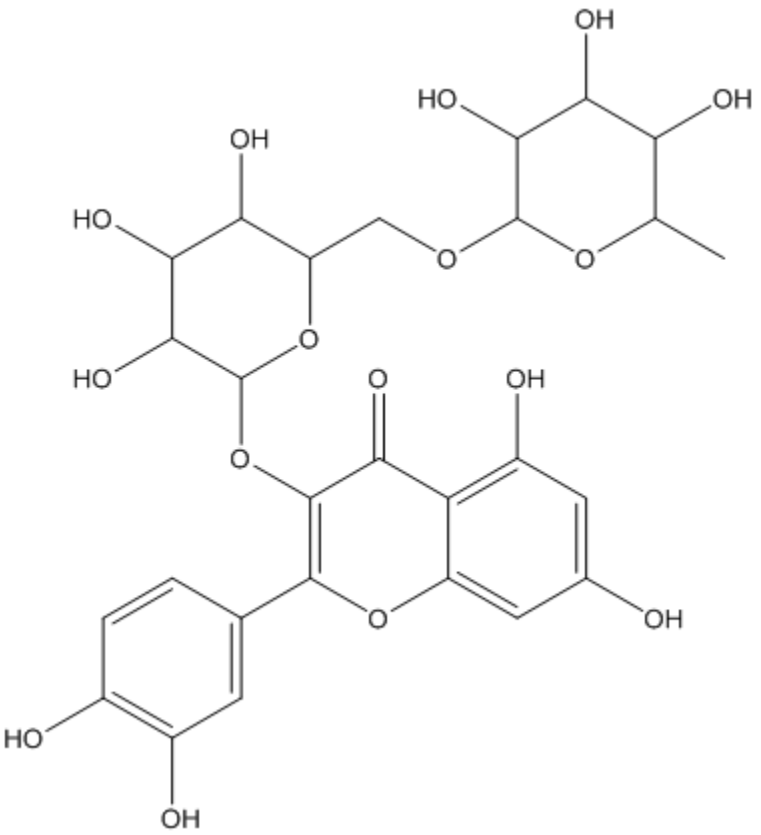


Figure 3j:- Rutin

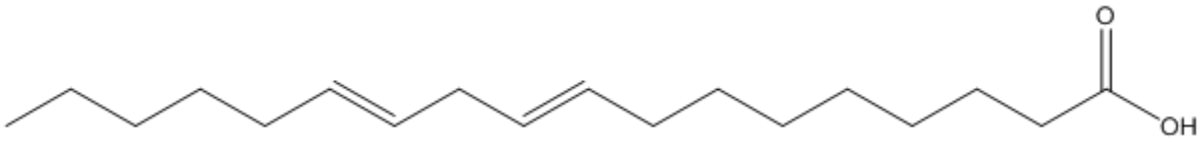


Figure 4a:-Linoleic Acid

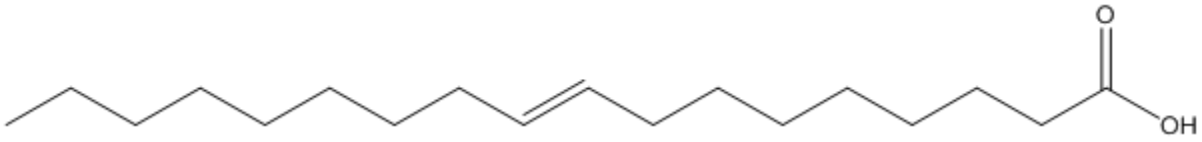


Figure 4b:- Oleic Acid

7. Materials and Methods:

7.1. Plant Material:

Leaves of *Tecoma stans* (TS1 and TS2) were collected from Santipur, Nadia, West Bengal, India and from Durgapur,Paschim Burdwan, West Bengal during the month of September. The plant was considerately identified and authenticated (Specimen No. **JU/DP-01**) by Central National Herbarium, Botanical Survey of India, Ministry of Environment, Foreign & Climate Change, Govt. of India, Howrah-711103.

TS1: *Tecoma stans* variety 1

TS2: *Tecoma stans* variety 2

7.2. Chemicals: Methanol, Glacial Acetic Acid

8. Extract Preparation:

8.1. Soxhlet extraction:

Leaves of both varieties of *Tecoma stans* were washed and shed dried at room temperature under fan. The dried leaves were pulverized into coarse powder by using mechanical grinder (Figure 5). After that the coarse powder of both varieties were extracted with the solvent methanol for 20 days each (3 to 6 hrs each day) by using Soxhlet apparatus (Figure 6). The liquid material from extraction was then concentrated by using rotary evaporator and the concentrated material was then freeze dried using lyophilizer. After that the extraction dried semi-solid material was then stored in the vacuum desiccator (Figure 7 & 8).

Figure 5: Powdered Plant leaves

Figure 6: Soxhlet Extraction

Figure 7: Extract 1(TS1)

Figure 8: Extract 2(TS2)

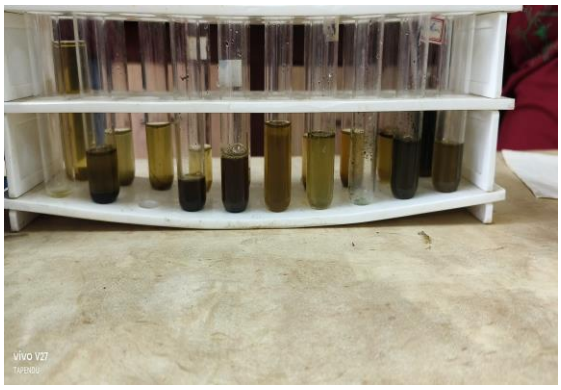


Figure 9: Phytochemical screening

9. Phytochemical Screening: Phytochemical screening was performed with the help of the standard procedures (Kokate *et al*.2019, Mandal *et al*.2015) (Figure 9).

9.1. Test for Carbohydrate:

(a) Molisch Test: At first the extract solution (2ml) was treated with Molisch reagent and to which 2ml of concentrated sulfuric acid was added. The formation of purple colour ring at the junction of the two layers indicates the presence of Carbohydrate.

(b) Fehling's Test: Combined equivalent amounts of extract and Fehling's reagent (equal quantities of Fehling's reagent A and B) and heated. The emergence of a rust-colored sediment suggests the existence of reducing sugar.

9.2. Test for Proteins and Amino acids:

(a) Biuret Test: Upon mixing 2 ml of extract with 2 ml of biuret solution the purple colour confirms the Presence of protein.

(b) Millon's Test: The extracted solution was mixed with 2ml of millon's reagent. If a white precipitate forms, it suggests the existence of amino acids.

9.3. Test for Steroids:

(a) Salkowski Test: An equal volume of extract and chloroform are exposed to a few drops of highly concentrated sulfuric acid. The existence of steroids is demonstrated by the appearance of a reddish-brown colour in the underlying layer.

(b) Liebermann Burchard Test: The extract solution was processed an initial treatment with acetic anhydride followed by the addition of a few drops of concentrated sulfuric acid along the edge of the test tube. The appearance of a green colour indicated the existence of steroids in the mixture.

9.4. Test for Alkaloid:

(a) Mayer's Test: Incorporating 2ml of extract with 0.2ml dilute HCl and then with a couple of drops of Mayer's reagent resulted in a yellow precipitate formation confirming the existence of alkaloids.

(b) Dragendorff's Test: A quantity of 2 ml of extract was combined with 0.1 ml of diluted hydrochloric acid and a small amount of Dragendroff's reagent. The emergence of an orange-brown precipitate indicates the existence of alkaloids.

(c) Wagner's Test: A volume of 2 ml of extract underwent the treatment with 0.1 ml of diluted hydrochloric acid followed by the addition of several drops of Wagner's reagent. The emergence of a reddish-brown precipitate indicates the presence of alkaloids.

9.5. Test for Glycosides:

9.5.1. Cardiac Glycosides:

(a) Keller-Kiliani Test: In a test tube 2 ml of extract was combined with glacial acetic acid along with a small amount of 5% Fecl3 and concentrated Sulfuric acid. The junction of the two layers displayed a reddish-brown ring while the top layer exhibited a bluish green colour indicating the existence of Glycosides.

(b) Legal's Test: The addition of 1 ml of sodium nitroprusside to an equal amount of Pyridine solution and extract resulted in a pink colour indicating the presence of glycosides.

(c) Foam Test: On shaking the extract vigorously with water which forms Stable foam indicates the presence of Saponin.

9.5.2. Anthraquinone Glycosides:

(a) Borntrager's Test: 2 ml of extract with diluted sulfuric acid was boiled for 5 minutes and proceeded to filtration. Then the filtrate was allowed to cool down before mixing it with an equivalent amount of chloroform. After that the chloroform layer was separated and mixed by shaking with half of its volume of diluted ammonia. The ammoniacal portion turned to red or pink colour indicating the presence of glycosides

9.6. Test for Flavonoids:

(a) Ferric Chloride Test: A few drops of 10 % $FeCl_3$ was added to the extract which forms a green colour. This indicates the presence of flavonoids.

(b) Sodium Hydroxide Test: Sodium Hydroxide was added to the extract which forms a yellow colour. This colour disappears after the addition of acid.

9.7. Test for Tannins and Phenolics:

(a) Gelatin Test: 1% Gelatin solution was added to the extract and was treated with 10% NaCl which forms a precipitate.

(b)FeCl3 Test: Upon treatment of the extract with 5% $FeCl_3$ solution, a dark green or bluish black colour appeared.

(c)10% NaOH Test: At first the extract was exposed to 10% NaOH and shaken vigorously which resulted the formation of emulsion. It indicates the presence of tannins.

10. GC-MS Analysis: Chromatographic separation was performed on a gas chromato-mass spectrometric system model (MassHunter GC/MS Acquisition 10.0.368 14-Feb-2019 Copyright

© 1989-2018 Agilent Technologies, Inc.) using a column DB-5MS (30mX0.25mmX0.25um). Other parameters are as following: Run time: 38 min, Injection volume: 1 uL, Inlet temperature: 280C, Inlet mode: Splitless, Solvent delay: 6 mins, MS Source temperature: 230C, MS Quad temperature: 150C. Detection was held in the SCAN mode in the range of (38–800 m/z). And the oven program is as follows:

*	*	**Temp.**	**Hold time**
*	*	40C	10 min
Rate 1	5C/min	180C	0s min
Rate 2	25C/min	280C	5 min

11.*In-vivo* Analysis:

11.1. Animals: Swiss albino mice (20–40 g) (either sex) were Purchased from M/S Chakraborty Enterprise (Laboratory Animal Supplier. CPCSEA Registered Under Ministry of Environment and Climate Change Govt. of India, Regd.No.1443/PO/Bt/s/11/CPCSEA). Animals were maintained in a room with controlled temperature (22 ± 2 ◦C) for 12 h light/12 h dark cycle with free access to food and water. Twelve hours before each experiment, animals received only water. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory

11.2. Tail-flick Test: The central mechanism of analgesic activity was assessed through the implementation of the tail immersion technique. This method involved exposing animals to thermal stimuli by submerging the tips of their tails in hot water. A designated section of the tail was marked and dipped into a water bath that was regulated at 52.5 ± 0.2 ◦C. The duration of time it took for the tail to be withdrawn from the hot water was recorded as the reaction time which was measured before treatment and 30 minutes after treatment up to 120 mins. Here the control group was fed saline as treatment and the standard group was fed ASA (Acetyl Salicylic Acid Effervescent Tablet 325mg), Test 1 group and test 2 group animals were fed *Tecoma stans* (200 mg/kg, both varieties of extract i.e. TS1 and TS2). To prevent any potential tissue, damage the

cut-off time for tail immersion was set at a maximum of 15 seconds. The maximum possible Analgesia was evaluated as follows:

11.3. Acetic Acid Induced Writhing Mice fasted overnight were divided into four groups of two animals each. The animals were then treated with Saline (10 ml/kg, p.o.); *Tecoma stans* (200 mg/kg, p.o.); and ASA (Acetyl Salicylic Acid Effervescent Tablet 325mg) (100 mg/kg, p.o.). Sixty minutes after treatment was carried out, mice were administered with acetic acid (1%, v/v in saline, 0.1 ml, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was then counted for 30 min at 5 ı ˙ interval (Ishola, I.O. *et al*.,2011).

$$\text{Inhibition (\%)} = \frac{\text{Number of Writhes [Control]} - \text{Number of Writhes [Treatment]}}{\text{Number of Writhes [Control]}}$$

12. Results:

12.1. Result of Soxhlet Extraction:

The amount of taken powdered leaves: 96 g

The amount of extracted material after drying 31.4 g (TS1, variety 1) (% yield: 32.70%)

The amount of extracted material after drying 31.09 g (TS 2, variety 2) (% yield: 32.38%)

12.2. Result of Phytochemical Screening:

SL. No	Phytochemical Constituent		Phytochemical Test	Extract 1(TS1)	Extract 2(TS2)
1	Carbohydrate		Molisch Test	-	-
			Fehling's Test	+++	++
2	Proteins and Amino acids		Biuret Test	+++	++
			Millon's Test	-	-
3	Steroids		Salkowski Test	++	+
			Lieberman Burchard Test	+++	+
4	Alkaloids		Mayer's test	++++	++
			Dragendorff's Test	-	+
			Wagner's Test	+++	++
5	Glycosides	Cardiac Glycosides	Keller Kiliani Test	-	+
			Legal's Test	-	-
		Anthraquinone Glycosides	Borntrager's Test	+++	+
		Saponin	Foam Test	-	+
7	Flavonoids		Ferric Chloride Test	+++	++
			Sodium Hydroxide Test	-	+
8	Tannins and Phenolics		Gelatin Test	-	-
			Ferric Chloride Test	++	+++
			10% Sodium Hydroxide Test	+	-

12.3. Result of GC-MS Analysis:

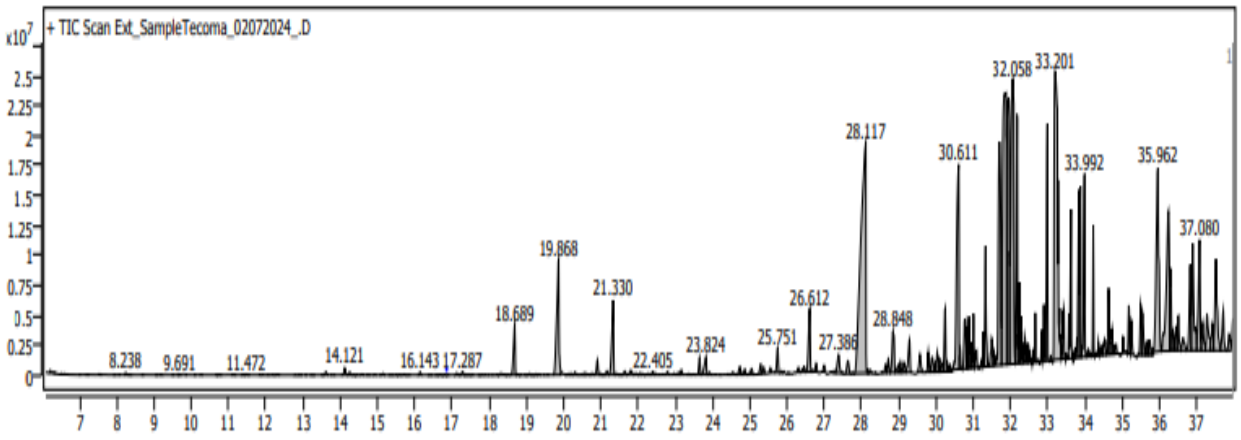
Sample_Tecoma_02072024			
Sr.No.	**Compound Name**	**Retention Time**	**Area**
1	4-Methoxy-1-butanol, TMS derivative	11.92	79593.84
2	Lactic Acid, 2TMS derivative	13.61	761345.53
3	Glycolic acid, 2TMS derivative	14.12	1669353.69
4	Sorbic acid, TMS derivative	15.83	52948.88
5	Hydracrylic acid, 2TMS derivative	16.14	581526.86
6	Benzoic Acid, TMS derivative	19.08	347939.1
7	Glycerol, 3TMS derivative	19.87	39402493.94
8	Butanedioic acid, 2TMS derivative	20.91	3448413.53
9	Glyceric acid, 3TMS derivative	21.33	19559651.97
10	Salicylic acid, TMS derivative	21.8	1669431.95
11	Erythrono-1,4-lactone, (E)-, 2TMS derivative	22.41	696689.35
12	3,4-Dihydroxy-5-methyl-dihydrofuran-2-one, (D)-, 2TMS derivative	22.96	283867.08
13	Erythrono-1,4-lactone, 2TMS derivative	23.82	5579547.46
14	Butanoic acid, 3,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	23.92	460947.82
15	Eugenol TMS	24.87	1106469.47

16	Malic acid, 3TMS derivative	25.3	2068076.89
17	L-Threitol, 4TMS derivative	25.55	825583.47
18	meso-Erythritol, 4TMS derivative	25.75	5396084.14
19	Erythronic acid, tetrakis(trimethylsilyl) deriv.	26.61	15965942.53
20	1,10-Decanediol, 2TMS derivative	28.12	177236556.5
21	Isopulegol, [1R-(1α,2β,5α)]-, TMS derivative	28.85	13043332.11
22	Azelaic acid, 2TBDMS derivative	30.61	71071874.55
23	Shikimic acid, 4TMS derivative	31.27	5727917.51
24	Quininic acid (5TMS)	31.7	79412926.4
25	D-Fructose, 1,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxime	31.88	155590855.6
26	D-Gluconic acid, 6TMS derivative	32.67	4431816.68
27	Palmitic Acid, TMS derivative	32.99	36982356.99
28	1,2,3,4,5,6-Hexa-O-trimethelsilyl-myo-inositol	33.2	154827867.3
29	Phytol, TMS derivative	33.63	25884099.92
30	α-Linolenic acid, TMS derivative	33.88	25852779.67

31	Stearic acid, TMS derivative	33.99	28025825.88
32	Sucrose, 8TMS derivative	36.3	16437187.02
33	Glycerol monostearate, 2TMS derivative	37.72	10504441.02

These are the list of prominent compounds present in the sample

12.3.1 Sample Chromatogram (GC-MS):



Counts vs. Acquisition Time (min)

Figure 10: GC-MS Diagram

:

12.4. Result of Tail Immersion Method:

Treatment	Reaction Time in Seconds (For Male)				
	0 min	30 min	60 min	90 min	120 min
Control (Saline)	0.632±0.1411	0.716±0.1562	0.764±0.1147	0.79±0.0947	0.826±0.0974
TS1 (200mg/kg)	0.662±0.0491	0.726±0.0214	0.864±0.0565	0.89±0.0126	0.966±0.0596
TS2 (200mg/kg)	0.682±0.1690	0.7965±0.054	0.925±0.244	0.9995±0.0564	1.326±0.0549
Standard ASA (100mg/kg)	0.6742±0.0497	0.826±0.0999	0.964±0.0458	1.089±0.0979	1.59926±0.0458

Values are Mean± SD

Treatment	Reaction Time in Seconds (For Female)				
	0 min	30 min	60 min	90 min	120 min
Control (Saline)	0.632±0.1411	0.672±0.1562	0.704±0.1148	0.712±0.0947	0.8003±0.0974
TS1(200mg/kg)	0.6562±0.0491	0.7003±0.0214	0.7996±0.0565	0.8012±0.0126	0.9006±0.0596
TS2(200mg/kg)	0.682±0.1690	0.7217±0.054	0.893±0.244	0.9099±0.0564	1.0326±0.0549
Standard ASA(100mg/kg)	0.674±0.0497	0.826±0.0999	0.9564±0.0458	1.089±0.0979475	1.326±0.0458

Values are Mean± SD

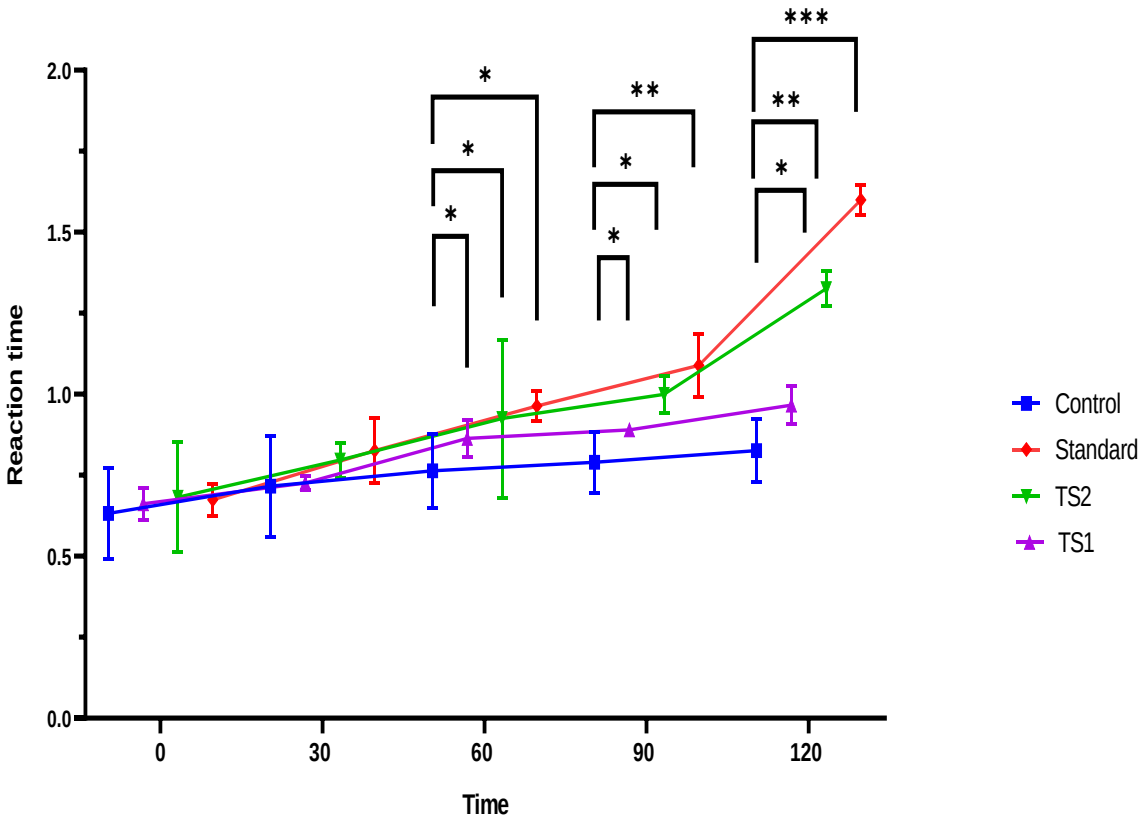


Figure 11: Graph of tail immersion Reaction time in respect of time (for male)

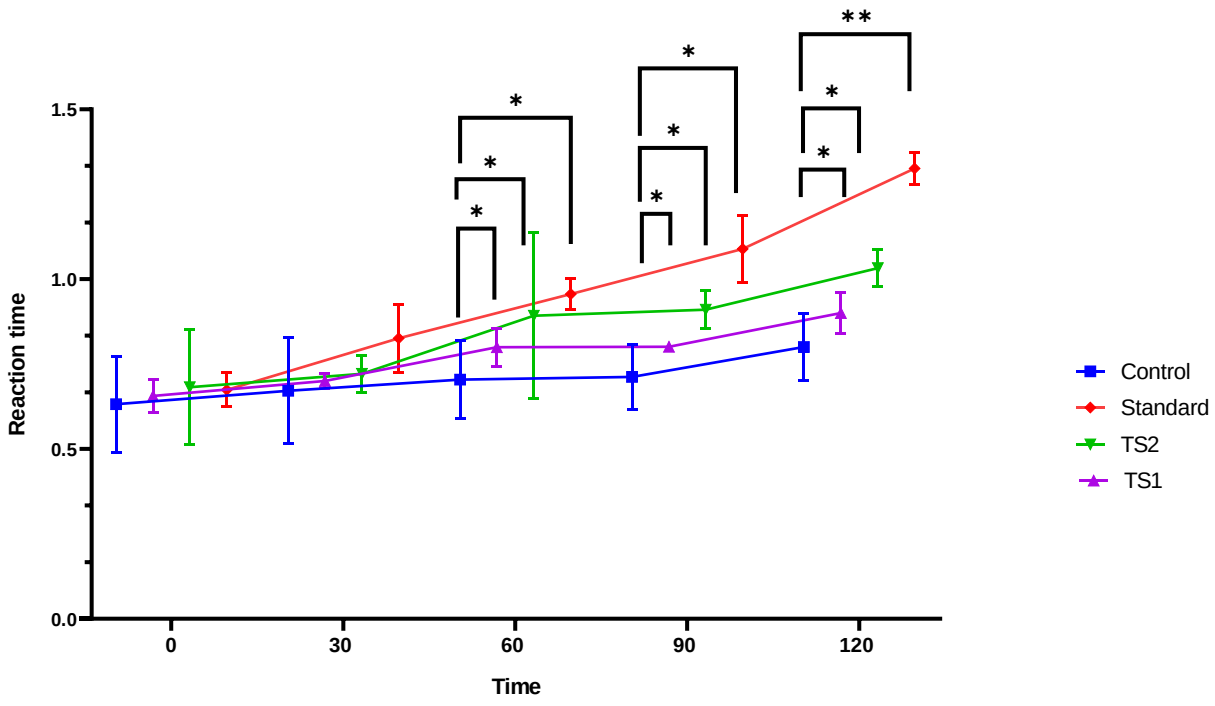


Figure 12: Graph of tail immersion Reaction time in respect of time (for female)

12.5. Result of Acetic Acid Induced Writhing:

Treatment	Dose(mg/Kg)	Total Number of Writhes	% Inhibition
Saline	10 ml/kg	23±1.871	-
TS1	200	20±1.581	13.04
TS2	200	18.80±1.924	18.26
ASA	100	17.20±1.304	25.21

Values are Mean± SD

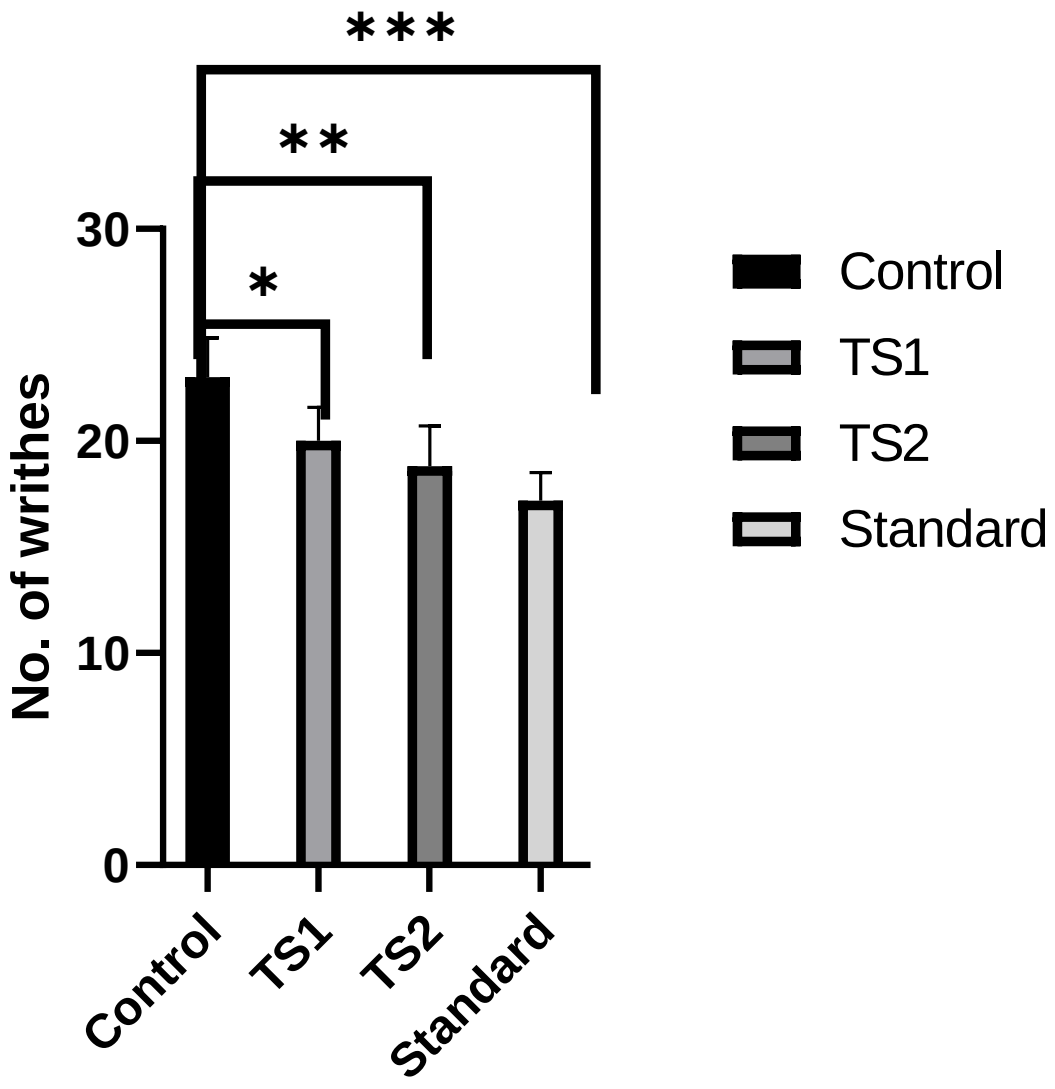


Figure 13: Acetic Acid Induce Writhing graph groups vs no of writhes in a time course
(* means adjusted p value 0.0326, ** means adjusted p value 0.0032,*** means adjusted p value0.0002)

13. Discussion:

Both the Varieties of the extracts showed activity in a dose dependent manner. The tail immersion test are widely used for assessing central antinociceptive activities. Furthermore, this test is distinguished by its tendency to respond to the pain stimuli conducting through neuronal pathways, as the tail immersion mediates spinal reflexes to nociceptive stimuli of PGE2. Both the varieties of extracts showed better activity in both groups of male and female mice but less than standard. It was found that TS2 ($p< 0.01$) is more active than TS1 ($p< 0.05$).

Acetic acid also induces sympathetic nervous system mediators, which are found in high level at first 30 min after acetic acid injection. This probably indicates that the analgesic activity of the extracts was mediated by inflammatory as well as neurogenic mechanisms. Similar value was found with TS2 ($p< 0.01$) compared with TS1 ($p< 0.05$) with dose-dependent decrease in the number of abdominal constrictions.

On phytochemical screening it was also found that TS2 possess more tannins and phenolic.

So overall it can be said that TS2 has better activity among the two varieties.

Further research will to isolate responsible phytoconstituents for proper pharmacological action.

14. Conclusion:

The research findings of these experiments indicate that the methanolic leaves extract of *Tecoma stans* collected from Durgapur, West Bengal has better efficacy compared to the plant present at Santipur, West Bengal. Santipur is in planar region whereas Durgapur area having rocky topography with laterite soil. It is also expected that plants from Durgapur area, contains properties that can relieve pain and reduce inflammation in better manner. These positive outcomes support the traditional use of this plant extract in treating conditions involving pain and inflammation.

The abundance of numerous phytochemicals found in methanolic extract of *Tecoma stans* leaves validates its traditional use by the nearby population as a medicinal plant and offers potential for the creation of new pharmaceuticals and nutraceutical products. Additional research on isolating and identifying the individual compounds could reveal their unique properties while investigating the combined effects of these compounds could enhance their efficacy in therapeutic applications. Further exploration in this area would be valuable in uncovering the full potential of *Tecoma stans*.

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