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CONVENTIONAL AND NON-CONVENTIONAL TECHNIQUES FOR EXTRACTION OF FLAVONOIDS FROM SELECTED PLANTS

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ABSTRACT: Medicinal plants are enriched with biological substances that are proven to be a direct or indirect source of modern drugs. The relevance of the exploration of new natural reservoirs of pharmaceutical potential has increased immensely after COVID-19. The present study deals with the extraction of flavonoids from selected plants i.e. *Ricinus communis*, *Euphorbia hirta* and *Croton bonplandinum* from the family Euporbiaceae by following conventional and non-conventional techniques. The conventional and non-conventional method includes Soxhlet extraction and Ultrasonic Assisted Extraction, respectively. Significant and comparable results were obtained for the ultrasonic technique in terms of the total quantity of extracts. The quantity of total flavonoid extract from the root, stem of *Ricinus communis* and stem, fruit, and root of *croton bonplandinum* is much greater in UAE (4.8mg/gdw and 36.9mg/gdw, respectively; 8.9mg/gdw, 60.5 g/gdw, 10.7 mg/gdw respectively) than Soxhlet method (0.95 mg/gdw and 4.8 mg/gdw respectively; 0.99 mg/gdw, 13.54 mg/gdw and 1.29 mg/gdw, respectively). The higher quantity of total flavonoids was recorded to be more for all extracts of *Euphorbia hirta* by the Soxhlet technique than the UAE method. The present investigation advocates the use of UAE as a regular extraction technique as it consumes less amount of solvent and plant sample than the Soxhlet technique. Moreover, it has been found more efficient and flexible in terms of time management and easy regulation of the protocol. The major constraint of Soxhlet extraction is the exorbitant use of solvent and time, whereas UAE offers reasonable and economical utilization of time and solvent.

INTRODUCTION: Humanity has used plant-based medicines to treat ailments for thousands of years. Novel chemical compounds with the potential as pharmaceutics and industrial can be found in plants. Alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenols, and flavonoids are only a few of the active substances found in plants, which are deposited in various sections of the plant such as the leaves, flowers, bark, seeds, fruits, roots, *etc* [1].

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For the isolation of biological components, extraction is one of the more sustainable approaches for plants [2]. Due to their unrivaled availability of chemical diversity, natural products such as plant extract, whether in the form of pure chemicals or standardized extracts, provide limitless prospects for the discovery of new drugs [3].

Extraction is the first and most crucial step in the analysis of medicinal plants since it is essential to distinguish and identify the necessary chemical components from the plant materials. The primary steps were pre-washing, freeze-drying, or drying plant materials, grinding to provide a homogenous sample, and frequently enhancing the kinetics of analytical extraction as well as increasing the contact of the sample surface with the solvent system.

In order to prevent possible active ingredients from being lost, altered, or destroyed during the extraction of plant samples, the appropriate steps must be done. Polar solvents like methanol, ethanol, or ethyl acetate are used to extract hydrophilic substances [4]. The qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction methods [5]. Extraction is the initial step in any study of a medicinal plant, and it is very important to the conclusion and result.

Different extraction techniques can be used to extract plant components. Non-conventional methods, which are more environmentally friendly due to the economical use of synthetic and organic chemicals, reduced operational time, and better yield and quality of extract, have been developed during the last 50 years [6]. It has been observed that unconventional techniques increase the overall yield and selectivity of bioactive components from plant sources. However, traditional extraction techniques like Soxhlet are still used as a benchmark to assess the efficacy of newer developed ones.

Conventional extraction techniques include a number of traditional methods such as maceration, hydro distillation and Soxhlet extraction. Maceration was used in the homemade preparation of tonic for a long time. It became a popular and inexpensive way to get essential oils and bioactive. Hydro distillation is a traditional method for the extraction of bioactive compounds and essential oils from plants. Water distillation, water and steam distillation, and direct steam distillation are the three different kinds of hydro distillation [7].

The Soxhlet extractor was first proposed by German chemist Franz Ritter von Soxhlet (1879). It is common practice to employ Soxhlet extraction to draw important bioactive chemicals from a variety of natural sources. A thimble is filled with a small amount of the dry sample. The distillation flask containing the solvent of particular interest is subsequently filled with the thimble. The solution of the thimble holder is sucked by a siphon whenever it reaches an excess level. The solution is siphoned back into the distillation flask. This mixture introduces extracts into the main liquid. The solvent is still present in the distillation flask and returns to the solid plant bed until the extraction is finished, and the operation is repeated. To avoid the shortcomings of traditional methods new innovative and efficient techniques for extraction must be explored. Longer extraction times, the need for expensive, high-purity solvents, the evaporation of enormous amounts of solvent, low extraction selectivity, and the heat breakdown of thermo-labile chemicals are the main drawbacks of conventional extraction [8].

New and promising extraction approaches are offered to address these limitations of traditional extraction techniques. These techniques are referred to as nonconventional extraction techniques. Some of the most promising techniques are ultrasound-assisted extraction [9], enzyme-assisted extraction [10], microwave-assisted extraction [11,] pulsed electric field-assisted extraction [12], supercritical fluid extraction [13] and pressurized liquid extraction.

Ultrasound-assisted Extraction (UAE): A unique class of sound wave that is audible to humans is ultrasound ranges from 20 kHz to 100 MHz it moves across a material by compressing and expanding, Cavitation is a phenomenon that results from this process and refers to the formation, expansion, and collapse of bubbles. The heating of the bubble's contents results from the conversion of the motion's kinetic energy, which can yield a significant quantity of energy**.** According to bubbles have a temperature of about 5000 K, a pressure of 1000 atm, and, a heating and cooling rate above 1010 K/s. UAE has been, developed on this principle.

The two primary physical processes involved in the ultrasonic extraction method are (a) diffusion over the cell wall and (b) rinsing the contents of the cell once the walls have been broken. The sample's moisture content, degree of milling, particle size, and solvent all play crucial roles in achieving an effective and efficient extraction. In addition, the parameters that control ultrasonic action are temperature, pressure, frequency, and period of sonication [14, 15]. The conventional method exhausts time, samples, and solvent for extraction with less quantity of extracts. In order to curb these limitations, new extraction techniques such as UAE should be explored regularly.

Optimization of extraction protocols using new, innovative and alternative ways that are frugal in the consumption of sample, solvent and time along with significant quantities of extracts is always welcome.

MATERIAL AND METHOD:

Collection and Authentication of Plants: Selected plants i.e., *Euphorbia hirta, Ricinus communis,* and *Croton bonplandinum* were collected from different areas and localities of Jaipur and Tonk, Rajasthan. A voucher specimen was submitted to the Herbarium, Department of Botany, University of Rajasthan, Jaipur, andauthentication was given to each plant.

(*Ricinus communis*: RUBL21204, *Euphorbia hirta*: RUBL21205, *Croton bonplandinum*: RUBL21280).

Different parts of selected plants were washed with tap water for the removal of dust and shade dried. Plant samples were then grounded into a fine powder and stored in airtight boxes for future use.

Preliminary Detection test for Flavonoids: Samples were subjected to preliminary detection tests for flavonoid aqueous extracts and powdered plants [16]. The following methods were used to determine the presence of flavonoids.

NaOH Test: The appearance of yellow color with addition of NaOH to the test sample indicates the presence of flavonoids in the sample.

Ethyle Acetate Test: A portion of the powered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min., the mixture was filtered and 1 ml of dilute ammonia solution was added to 4 ml of filtrate. A yellow coloration was observed, indicating a positive test for flavonoids. Five ml of diluted ammonia solution was added to the aqueous plant extract, followed by the addition of con. H2So4 yellow color was observed in each extract, indicating the presence of flavonoids. The Yellow color disappeared on standing.

Schinoda Test: to each test sample a piece of magnesium ribbon and concentrated HCl was added dropwise. A pink, scarlet, crimson, or occasionally green or blue color indicated the presence of flavonoid.

Extraction of Flavonoids: Soxhlet extraction was followed as a conventional method for extracting flavonoids from different parts of selected plants. 100 grams of a finely powdered sample of each plant part was Soxhlet extracted with 80% of methanol on a water bath for 24 hours keeping the plant powder sample and methanol ratio 1:2. After 24 hours sample was filtered. Ultrasound-assisted extraction (UAE) was followed as a non-conventional extraction technique, where ultrasonic/ultrasound waving was given to release different Phyto compounds from plant matrices. 10 grams of a finely powdered sample of each plant was subjected to extraction to UAE in the ultrasonicate bath for 15 minutes at 60 °C method was optimized by using varied temperatures and time along with the quantity of solvent used.

The result was stable at a temperature 60°C at a time of 15 minutes. In both methods, different plant parts of *Euphorbia hirta, Ricinus Communis,* and *Croton bonplandinum* were subjected to flavonoid extraction [14]. The sample was filtrated and extracted successively with petroleum ether, ethyl ether, and ethyl acetate. Petroleum ether fractions were discarded due to being rich in fatty substances whereas ethyl ether and ethyl acetate fractions were analyzed for free flavonoids and bound flavonoids. The ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% of sulfuric acid respectively for 2 hours. The resulting mixture was filtered and washed with distilled water to neutrality. Ethyl ether and ethyl acetate extracts are abbreviated as E1 and E2 respectively. All flavonoid extracts were dried, weighed, and stored at 4 °C in air-tight glass vials

RESULT: Free, bound, total flavonoids from different parts of selected plants were isolated, and quantity mg/gdw was calculated for each plant extract. Total flavonoid content from the stem, leaf, fruit, and root of *Ricinus communis* obtained was 0.95mg/gdw, 6.37mg/gdw, 46.4 mg/gdw, 15.25 mg/gdw respectively in the conventional method. total flavonoids obtained was 4.8 mg/gdw, 1.4 mg/gdw, 23.5 mg/gdw 36.9, mg/gdw from stem, leaf, fruit, and root respectively. It is noteworthy that a significant difference in the quantity of total flavonoid from root and stem was recorded for the UAE method than the classical method **(Fig. 1, Fig. 2** and **Table 1).**

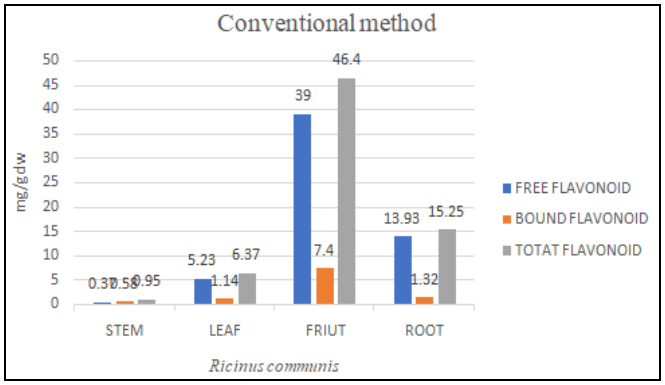

FIG. 1: QUANTITY OF FLAVONOIDS FROM *RICINUS COMMUNIS* FROM CONVENTIONAL

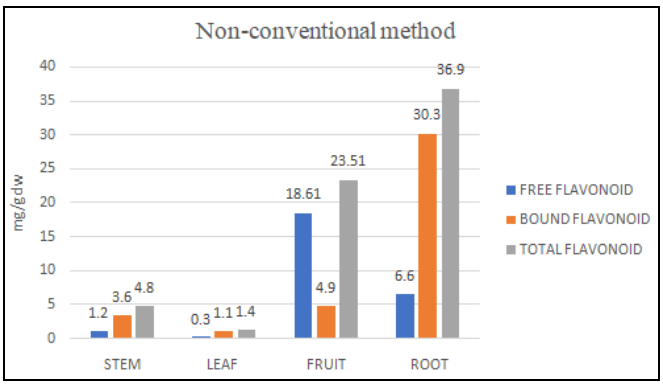

FIG. 2: QUANTITY OF FLAVONOIDS FROM *RICINUS COMMUNIS* FROM NON-CONVENTIONAL METHOD

In the conventional method, total flavonoid content obtained from stem, leaf, fruit and root was 81.98 mg/gdw, 67.07 mg, /gdw, 14.42 mg/gdw and 5.93 mg/gdwin *Euphorbia hirta* respectively. Total flavonoid content obtained in the non-conventional method was 3.5 mg/gdw, 7.5mg /gdw, 0.7 mg/gdw, and 2.6 mg/gdw respectively. It is noted that a significant difference in the quantity of total flavonoid from stem, leaf, fruit, and root was recorded for the conventional method than the UAE method (**Fig. 3, Fig. 4** and **Table 1**).

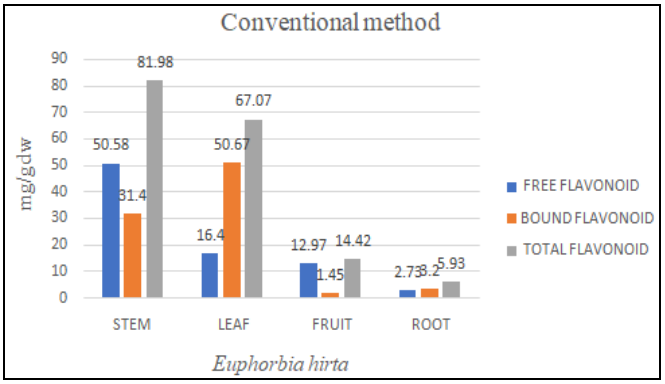

FIG. 3: QUANTITY OF FLAVONOIDS FROM *EUPHORBIA HIRTA* BY CONVENTIONAL METHOD

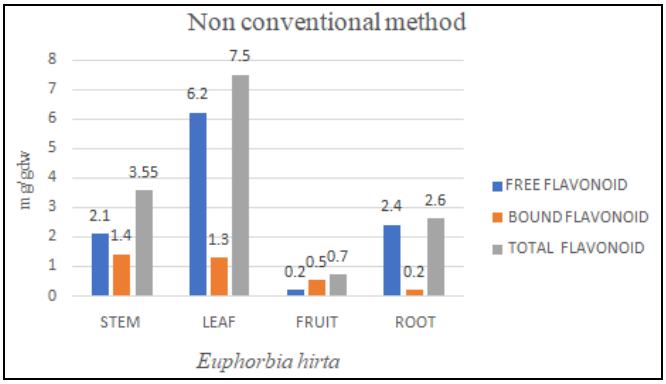

FIG. 4: QUANTITY OF FLAVONOIDS *IN EUPHORBIA HIRTA* BY NON-CONVENTIONAL METHOD

In *Croton bonplandinum* total flavonoid content obtained from stem, leaf, fruit, and root was 0.99mg/gdw, 14.22 mg/gdw, 13.54 mg/gdw, 1.29 mg/gdw in the conventional method respectively. In the non-conventional method, total flavonoid obtained 8.9mg/gdw, 11.1 mg/gdw, 60.5mg/gdw, and 10.7 mg/gdw respectively. It is noteworthy that a significant difference in the quantity of total flavonoid from stem, fruit and root was recorded for the UAE med than the traditional method **(Fig. 5, Fig. 6 and Table 1).**

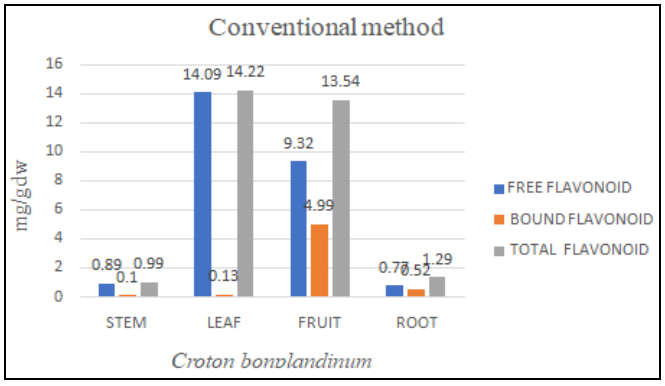

FIG. 5: QUANTITY OF FLAVONOIDS FROM *CROTON BONPLANDINUM* BY CONVENTIONAL METHOD

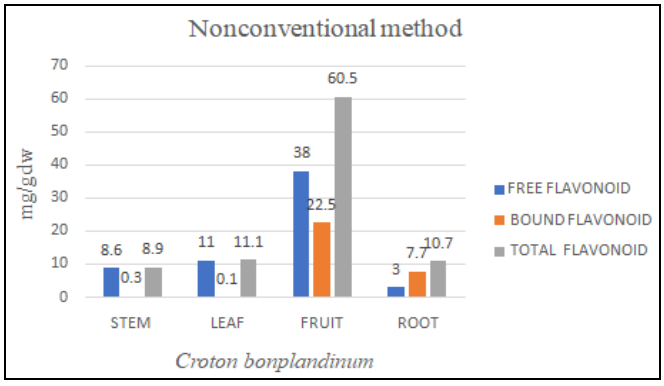

FIG. 6: QUANTITY OF FLAVONOIDS FROM*CROTON BONPLANDINUM* BY NON-CONVENTIONAL METHOD

TABLE 1: AMOUNT OF METABOLITES WITH THEIR COLOUR APPEARANCE OF DIFFERENT PLAN PARTS OF *RICINUS COMMUNIS*, *EUPHORBIA HIRTA*, AND *CROTON BONPLANDINUM* OBTAINED BY CONVENTIONAL AND NON-CONVENTIONAL METHODS

S. no.	Plant Name	Plant Part	Conventional Methods (Soxhlet)					Non- conventional Methods UAE 60 ^{0}C for 15 min				
			Ethyl ether (EE), Free Flavonoids (E_1) mg/gdw	Color Appearance E_1	Ethyl Acetate (EA) Amount of Bound Flavonoid (E_2) mg/gdw	Color Appearance of E_2	Total Flavonoid	Ethyl ether (EE), Free Flavonoids (E_1) mg/gdw	Color Appreance of E_1	Ethyl Acetate (EA) Amount of Bound Flavonoid (E_2)mg/gdw	Color Appearance E_2	Total Flavonoid
1	*Ricinus communis*	Stem	0.37	Light green	0.58	Yellow	0.95	1.2	Yellow	3.6	Light green	4.8
		Leaf	5.23	Dark green	1.14	Green	6.37	0.3	Light green	1.1	Dark-green	1.4
		Fruit	39	Dark green	7.4	Yellow	46.4	18.6	Transparent	4.9	Light green	23.5
		Root	13.93	Brown	1.32	Light brown	15.25	6.6	Dark Yellow	30.3	white	36.9
2	*Euphorbia hirta*	Stem	50.58	Light green	31.4	Dark brown	81.98	2.1	Light green	1.4	Light green	3.5
		Leaf	16.4	Dark green	50.67	Light green	67.07	6.2	Dark green	1.3	Dark-green	7.5
		Fruit	12.97	Light green	1.45	Light brown	14.42	0.2	Light green	0.5	Light green	0.7
		Root	2.73	Light yellow	3.2	Slightly pink	5.93	2.4	Brown	0.2	Light green	2.6
3	*Crotanbon plaandium*	Stem	0.89	green	0.1	Brown	0.99	8.6	Green	0.3	Brown	8.9
		Leaf	14.09	Dark green	0.13	Brown	14.22	11	Dark green	0.1	Yellow	11.1
		Fruit	9.32	green	4.99	Dark brown	13.54	38	Dark green	22.5	Green	60.5
		Root						3	Yellow	7.7	Yellow	10.7

When extracting the stem, Leaf, Fruit, and root of *Ricinus communis*, *Euphorbia hirta*, and *Crotnbon plandinum* by conventional and non-conventional methods, these three plants obtained different, different highest amounts of total flavonoids. The highest amount of total flavonoids from the stem and leaf of *Euphorbia hirta* (81.98mg/gdw and 67.07mg/gdw), Fruit of *Crotn bonplandinum* showed the maximum amount (60.5mg/gdw). While the root of *Ricinus communis* exhibited a maximum quantity of total flavonoids (36.9mg/gdw) among all extracts from selected plants. Significant increases in the number of extracts from *Crotn bonplandinum* were observed in almost all extracts obtained by UAE than the Soxhlet method. The UAE method was observed to be more efficient than the conventional Soxhlet method for five extracts among total of 12 extracts total flavonoids where a high quantity was obtained in less time and solvent. Although, Fruit of *Ricinus communis* and *Euphorbia hirta* gave a good quantity of total flavonoids by Soxhlet extraction by showing high amounts **(Table 2, Fig. 7)**.

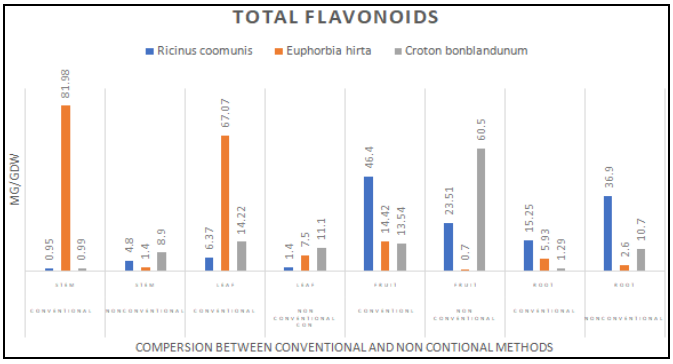

FIG. 7: COMPARISON BETWEEN CONVENTIONAL AND NONCONVENTIONAL METHODS

TABLE 2: COMPARISON BETWEEN CONVENTIONAL AND NONCONVENTIONAL METHODS

Total flavonoid	Conventional	Nonconventional	Conventional	Non-conventional Con	Conventional	Non-conventional	Conventional	Nonconventional
	Stem	Stem	Leaf	Leaf	Fruit	Fruit	Root	Root
Ricinus coomunis	0.95	4.8	6.37	1.4	46.4	23.5	15	36.9
Euphorbia hirta	81.98	1.4	67.07	7.5	14.4	0.7	5.9	2.6
Croton bonblandunum	0.99	8.9	14.22	11.1	13.5	60.5	1.3	10.7

CONCLUSION: In the present investigation, both techniques were found to be equivalent in terms of the quantity of extracts obtained from selected plants. Higher quantities of extracts from plant parts were recorded for UAE when Soxhlet same a much smaller quantity of the same extracts and vice versa. Although quantity findings were comparable for both techniques UAE was found to be more efficient, time-saving, and economic and solvent consumption, a more controllable technique than the conventional Soxhlet method. Optimization of UAE protocol may be done for the flavonoid extract, obtained in less quantity. Therefore, the present study advocates the use of modern techniques i.e., UAE for the extraction of different metabolites from plants. The discovery and identification of new therapeutic chemicals rely heavily on medicinal plants. Different phytochemicals from plants can be separated out and characterized using different extraction methods. But in this research, we found that conventional procedures are laborious, time-consuming, expensive, require high-purity solvent, evaporate enormous amounts of solvent, have low extraction selectivity, and consume more power, sample, and solvent. Traditional herbal treatments are progressing in development thanks to modern extraction techniques. Utilizing unconventional methods results in new and intriguing strategies that are frequently complementary to traditional methods. that the measurement of extraction efficiency is influenced by the proper use of standard methodologies**.**

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CONFLICT OF INTEREST: Nil

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