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Efficiency of Different Solvents and Extraction Methods for Urease Inhibition and per cent Yield of *Delonix regia* Extracts

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ABSTRACT

Urease is an enzyme that belongs to the super family of amidohydrolases and phosphotriesterases which is responsible for various gastrointestinal diseases including urinary stones. Various plant species were exploited for naturally inhibiting the urease enzyme. *Delonix regia* is traditionally known for its anti-ulcer, cytotoxic and antifungal activities. In this context, the present work was aimed at comparing the effect of four extraction processes (maceration, Soxhlet extraction, microwave-assisted extraction and ultrasound-assisted extraction) and five solvents (distilled water, methanol, acetone, dichloromethane and diethyl ether) on the extraction yield and urease inhibition potential of stem and leaf extracts of *D. regia*. The study showed that Soxhlet extraction process with polar solvents (distilled water and methanol) provided highest extraction yield (29.28% with leaf, and 13.88% and 13.78% with stem), while maceration and methanol exhibited highest urease inhibition potential (52.53±0.53% with stem and 55.28±0.37% with leaf) showing synchrony near to the standard inhibitor used i.e. thiourea with 73.58±0.02% inhibition activity. The work indicated that polar solvents were more significant for extraction and *D. regia* extracts were found to be promising natural urease inhibitor.

Key words: *Delonix regia*, extraction methods, solvents, per cent yield, urease inhibition

INTRODUCTION

Delonix regia (Gul Mohr), famous as flame tree, a flowering plant belongs to the family Fabaceae, sub-family Caesalpinioideae, popular for having fern like leaves, displaying flamboyant pattern of flowers and endemic to Madagascar. It is named as Royal Poinciana or Flamboyant in English and grown as an ornamental tree (Modi *et al.*, 2016). It is distributed in many tropical countries along with various parts of India, Africa and North Australia. Its height reaches up to 40 feet with large flowers having four spreading orange-red petals and having doubled pinnate leaves with 20-40 pair of primary and 10-20 pair of secondary leaflets. Its various parts such as leaves, bark, stem and flowers contain bioactive compounds and known for anti-helminthic, anti-inflammatory, anti-ulcer, cytotoxic and antifungal activities. Traditionally, its leaves are used for pneumonia in infants, bronchitis, gastric problems, anti-diabetic and rheumatic joint pains. *D. regia* has phytochemicals such as sterols, triterpenoidal saponins, flavinoids, organic acids, etc. (Jayanthi and Amoghimath, 2018).

Urease is an enzyme that belongs to super family of amidohydrolases and phosphotriesterases having nickel in its active site. Urease is meant to catalyze urea hydrolysis process which release ammonia that neutralize the acids and eventually results in growth of microorganisms (Saeed *et al.*, 2017). It has achieved a considerable attention for its impact on health and life of human beings. Urease is responsible for various diseases like gastritis, duodenal, peptic ulcer and gastric cancer, urolithiasis (Phull *et al.*, 2018), ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation, Parkinson's disease, urinary stones, pyelonephritis, etc. (Mahernia *et al.*, 2015; Kataria and Khatkar, 2019).

Plants having bioactive compounds are used for medicinal purpose. According to reports of World Health Organization (WHO) the use of medicinal plants and plant-based natural products in food, pharmaceutical and cosmetic industry had increased over years all over the world (Khan *et al.*, 2019; Drinic *et al.*, 2020).

The main stage of separation and utilization of bioactive compounds is extraction. Physical methods of extraction generally results in lower yield and higher energy consumption in contrast to chemical extraction (Saini and Keum, 2018). There are many parameters other than extraction methods that are responsible for alteration in extraction yield and biological activity of the plant extracts in industrial process like as particle size, solvent composition, initial preparations, solvent type, extraction temperature and pressure, solid to solvent ratio, pH and extraction time (Mohammadpour *et al.*, 2019).

Traditional extraction methods mostly utilize a larger amount of solvents and manual procedures that mostly depend on the researcher, requiring a long period for extraction, have health and environmental risks, and may also alter the properties of the extracted material. So, these are not ideally consistent (Alara *et al.*, 2018a, b). Since there are some drawbacks of using traditional methods, it is important to prevail over such challenges. Unconventional methods have been generated to fill the missing gap of the conventional methods (Shams *et al.*, 2015). Modern methods of extraction have some improved characteristics which include higher extraction efficiency, enhanced selectivity, automation, and lesser consumption of solvents, providing high-quality products with minimum risks and better advantages (Azwanida, 2015; Alara *et al.*, 2018a, b). Few advanced extraction methods have emerged for plant component extraction (Alara *et al.*, 2021). Besides all these advantages of using modern methods, conventional methods are mainly used for general extraction. It may be due to their cost effectiveness and easy handling.

Crude extracts generally have a group of different classes of phytochemicals that are soluble in solvent used in the extraction method. Extracts are commonly prepared using solvent extraction method which include conventional/traditional methods like maceration, percolation, decoction, digestion, infusion, Soxhlet extraction and serial exhaustive extraction, while unconventional/ advanced/modern methods such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical CO_2 extraction (SC–CO_2), pressurized fluid extraction (PFE), enzyme-assisted extraction (EAE) and a combination of methods have also been used for phytochemical extraction (Selvamuthukumaran and Shi, 2017; Alara *et al.*, 2021).

The most important factor for extraction is the type of solvent as its effects intersect the whole process, including the solubility of the target components to the extraction efficiency (yield as well as activity). Hence, one should include factors like solvent polarity, solvent power, solvent reactivity, boiling temperature of the solvent, solvent stability, solvent viscosity, potential reusability, legislature compatibility for usages and safety concerns, etc. during the selection of any solvent for extraction methods (Alara *et al.*, 2021). Non-polar substances can be extracted using non-polar solvents like hexane, petroleum ether, diethyl ether, etc. while polar solvents were meant for extracting polar phytochemicals (Roopa *et al.*, 2021).

As *D. regia* was known to be used for anti-ulcer, gastric problems, anti-diabetic activities, but it seems that its urease inhibition activity which may be responsible for hindering urinary and kidney stone formation is yet to be investigated. The positive results of the analysis may relevantly add new medicinal products in pharmaceutical industry against the kidney/urinary stone. This study was conducted to fulfill the aim of comparing the effect/alteration caused due to different extraction solvents (distilled water, methanol, acetone, dichloro methane and diethyl ether) and extraction methods {traditional (maceration and Soxhlet extraction) and modern methods (microwave-assisted extraction and ultrasound-assisted extraction)} on the extraction yield and urease inhibition activity of the *D. regia* extracts.

MATERIALS AND METHODS

The plant species (*D. regia*) of a family Fabaceae were selected based on their pharmacological reports and traditional uses. The leaves and young stem of the *D. regia* were collected in the months of August-September, 2019 from the campus of Kurukshetra University, Kurukshetra, India and were identified by Prof. B. D. Vashistha, Department of Botany, Kurukshetra University. A voucher specimen (Herbarium/Botany/KUK/Biotech 2019-2) of *D. regia* was deposited in a herbarium. The

plant materials were washed and rinsed with distilled water repeatedly to remove any soil or solid particulates and shade-dried at 40°C for 5 days. After drying, the sample was ground to a fine powder using a mechanical blender and stored for further use, before extraction.

Urease [Type IX from *Canavalia ensiformis* (Jack Bean) of specific activity: 50,000-100,000 units/g], one unit of urease enzyme equivalent to 1.0 I.U., other chemicals such as urea, thiourea, sodium nitroprusside, sodium hypochlorite, sodium hydroxide and phenol were purchased from Hi-Media. Solvents like diethyl ether, dichloro methane, acetone and methanol were also purchased from Hi-Media. All reagents were of analytical grade.

The Coarsely powdered leaves and young stem (each 10 g) were soaked in 100 ml of different solvents (diethyl ether, dichloro methane, acetone, methanol, distilled water) in separate reagent bottle for each sample and covered the lid at 37°C for 72 h. The content was stirred periodically (time to time) for ensuring complete extraction. At the end, the extracts were separated and filtered and evaporated to dryness in oven or rotary evaporator at 50°C. The extracts were dried and stored at -4 °C for further use (Kumar *et al.*, 2022).

Soxhlet extraction is also known as continous hot extraction. This process is carried out in an apparatus known as Soxhlet extractor which is made up of glass. It is comprised of a round bottom flask, condenser at the top, siphon tube and extraction chamber. 10 g of plant material (dried, grinded and finely powdered) was placed in crucibles inside the extraction chamber, bottom flask was poured with 100 ml of solvent and thimble was set over extraction chamber. The heating element of the Soxhlet allowed the solvent in the bottom flask to evaporate and pass through condenser which condensed and passed the solvent to extraction chamber and extracts on coming in contact with the plant material. Consequently, the level of solvent reached to the top of siphon, it flowed back to the bottom flask along with the extracted plant material. The process continued repeatedly until extraction was over (approx. 6 h). The point of completion was estimated as no colour or transparent solvent was siphoned out. The solvent and the extracted material collected in the bottom flask were filtered and subjected to rotary evaporator at 50°C for evaporation of solvent to dryness. The extracts were dried and stored at -4 °C for further use (Hirondart *et al.*, 2020).

Microwave-assisted extraction is a modern type of extraction technique. It uses the microwave radiations for bombardment to an object which absorb electromagnetic energy and converts it into heat. 10 g finely powdered leaves and stem material was poured in the closed bottles and 100 ml of solvent was added to the bottle. The cap was closed and the vessel was subjected to ONIDA POWER SOLO 17 D microwave oven at high power and 700 W voltage. The material was heated due to microwaves and extraction was allowed for 5-10 min. After completion, the bottle was removed from the microwave oven and allowed to cool. The extracted material was then filtered and evaporated using rotary evaporator at 50°C. The extracts were dried and stored at -4°C for further use (da Rocha and Norena, 2020; Bagade and Patil, 2021).

Ultrasound-assisted extraction involves application of sound energy at a very high frequency to disrupt the plant cell wall and increases the surface area for solvent penetration as well as extraction. The plant samples (10 g each) were poured in a 250 ml beaker, 100 ml of solvents were added and the whole mixture was subjected for ultrasonication using Hielscher ultrasound technology, UP200S, made in Germany sonicator at 0.5 cycles and 100% amplitude for 25 min. After completion of sonication, the extracted material was subjected to filtration and evaporation using rotary evaporator at 50°C. The extracts were dried and stored at -4°C for further use (Raj and Dash, 2020; Karbuz and Tugrul, 2021).

The dried extracts obtained using various solvents and methods were weighed to determine the percentage yield of each extract using following (Nofita *et al.*, 2022) as:

> Percentage extract yield (w/w) = 100 x [Weight of extracted plant material obtained (g)]/[Weight of plant material taken for extraction (g)]

The dried extracts were reconstituted to phosphate buffer for further analysis and urease inhibition studies.

The extracts obtained using different solvents and methods were dissolved in 1 ml of phosphate buffer making a concentration of 1 mg/ml. The assay buffer of pH 8.2 was prepared

using 10 mM phosphate buffer containing 10 mM $LiCl_2$ and 1 mM EDTA (ethylene diamine tetra acetic acid) and stored at 37°C. Urease from Sigma Aldrich Company was dissolved to 1 mg/5 ml concentration in phosphate buffer. Solution A (phenol reagent) was prepared using 1 g of phenol and 5 mg of sodium nitroprusside dissolved in 100 ml of sterile distilled water. While solution B (alkylating agent) comprised of 0.5 g sodium hydroxide and 840 µl of 5% sodium hypochlorite solution was dissolved in 100 ml of sterile distilled water. Urea of 30 mM concentration was used as substrate for the assay reaction. Thiourea was used as a standard inhibitor with concentration 1 mg/ml. For the inhibition assay, assay mixture comprising 1.2 ml assay buffer of pH 8.2 at 37°C, 0.2 ml (1 mg/5 ml) of urease enzyme solution and 0.1 ml of each sample was subjected to 5 min incubation. 0.5 ml of urea solution was added to the assay mixture and incubated at 25°C. After 20 min of incubation, 1 ml of freshly prepared solution A and solution B were added to the reaction mixture. After 30 min of incubation at 25°C, the urease activity was calculated by measuring absorbance of the released ammonia during the reaction at 640 nm wave length, using modified Weatherburn method (Irshad *et al.*, 2022).

$$I\% = 100 - (T/C \times 100)$$

Where I% denoted to the percentage inhibition of the enzyme, T (test) denoted to the absorbance of the tested sample in the presence of enzyme, C (control) denoted to the absorbance of without any inhibitor in the presence of enzyme.

All the experiments/assays were carried out in triplicates for testing the reproducibility of the assays. The results were presented as mean ±S.E.M. SPSS 15.0 was used for statistical analysis. The values of $P<0.05$ were considered statistically significant. Correlations among data obtained were calculated using Pearson's coefficient (r).

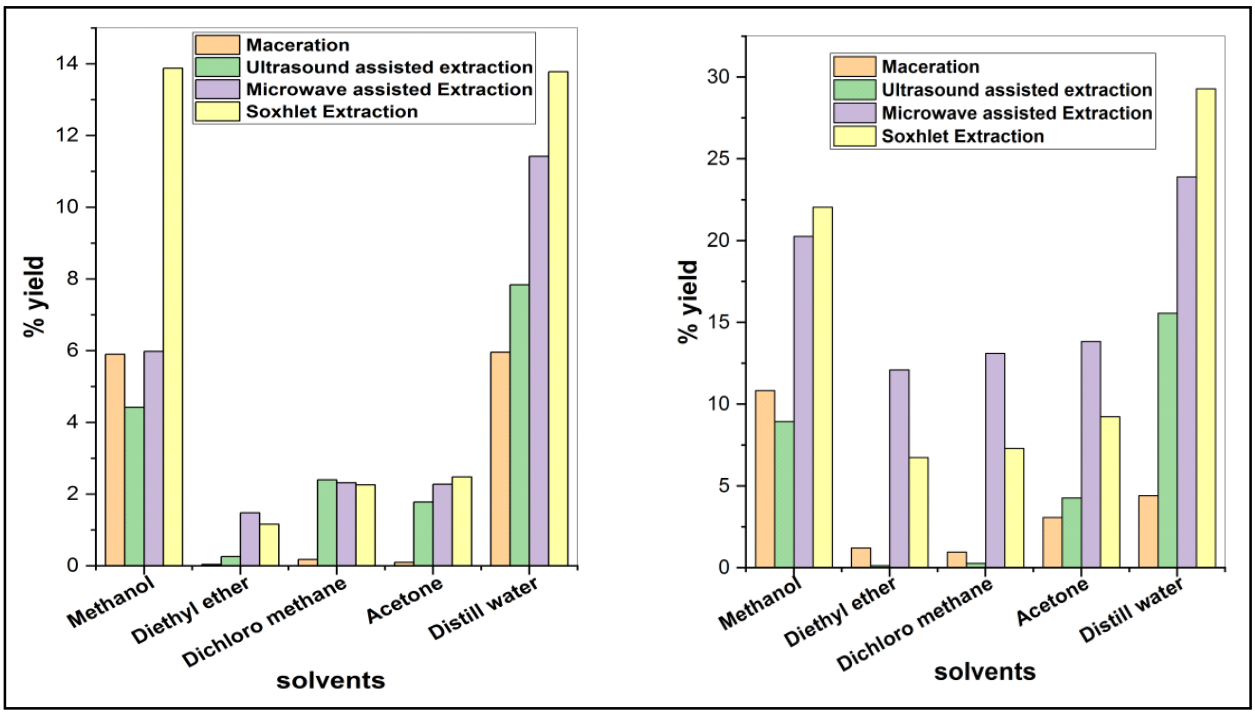
RESULTS AND DISCUSSION

Bioactive compounds are commonly present in plants but in meager concentration. Suitable extraction technique, efficient in extracting high yield of the bioactive components from plant extracts with enhanced activity and without disrupting the active functional properties is highly desirable (Wen *et al.*, 2018). Various studies have reported the efficiency of different extraction methods in regard to yield and biological activities of extracts. The selection of suitable solvent and extraction method usually depend on sample matrix properties, matrix analyte interaction, efficiency, chemical properties of the analyte and desired properties (Dhanani *et al.*, 2017). The extraction efficiency of the solvents depends mainly on the solubility of compounds in a particular solvent, mass diffusion rate, strength of interaction between solute and solvent and mass transfer kinetics of the compounds (Zia *et al.*, 2022). In recent extraction processes, the physical and chemical properties of materials are subjected to microwaves or ultrasonic wave treatments that altered their characteristics due to propagation and interaction of waves which disrupt the cell wall of materials and resulting into release of bioactive compounds (Picot-Allain *et al.*, 2022). The process involves the enhancement of mass transfer of solvents into plant cells. Microwaves and solvents are used for targeted extraction of bioactive component from plant cells using highly specific temperature and pressure conditions which causes the selective migration of targeted molecules from cell space to surrounding at a rapid rate (Daud *et al.*, 2022). Although, vast literature is available for the extraction of bioactive components using conventional solvents and extraction process yet a combined and detailed study related to it is not presented. In this context, present investigation focused on analyzing the efficiency of different solvents for extracting bioactive compounds, their yield and urease inhibition activity using different extraction processes. Further, on the basis of results the comparison between the solvents and process was made and results were presented.

The extraction yield of stem was found to be low in non-polar solvents like diethyl ether, dichloro methane and acetone in comparison to the polar solvents such as methanol and distilled water (Table 1 and Fig. 1). It may correspond to the presence of more quantity of polar compounds in the stem of *D. regia*. The results correspond to the previous reports in different plant species where the extraction yield was higher in the polar solvents as

Table 1. Effect of stem extracts of *D. regia* on per cent yield using different solvents and extraction methods

S. No.	Samples (plant part)	Solvent	Per cent yield			
			Maceration	Ultrasound-assisted extraction	Microwave-assisted extraction	Soxhlet extraction
1.	Stem (young)	Methanol	5.90	4.42	5.98	13.88
		Diethyl ether	0.04	0.26	1.48	1.16
		Dichloro methane	0.18	2.40	2.32	2.26
		Acetone	0.10	1.78	2.28	2.48
		Distilled water	5.96	7.84	11.42	13.78



(a) Stem extract (*D. regia*) **(b) Leaf extract (*D. regia*)**

Fig. 1. Graph showing comparison of % extraction yield using different methods and solvents.

compared to the non-polar solvents (Tan *et al.*, 2020). The presence of different phytochemicals and their functional groups is responsible for this variation in the extraction yield using different solvents. A difference in the polarity index of the water and the methanol is also responsible for the big difference in the extraction yield percentage using different extraction methods, although both are the polar solvents. Thus, the polar solvents resulted in increased extraction of the phytochemicals giving a higher extraction yields (Nawaz *et al*, 2020).

On studying the different processes, the Soxhlet extraction method yielded highest, using methanol i.e. 13.88% followed by distilled water (13.78%). The extract yield was found to be 02.48, 02.26 and 01.16%, using solvents acetone, DCM, DEE, respectively. The distilled water was found to be the best solvent if the microwave-assisted extraction process was used. The extract yield by microwave-assisted extraction process using distilled water was found to be11.42% followed by methanol (05.98%), DCM (02.32%), acetone (02.28%) and DEE (01.48%). Another extraction method i.e. ultrasound-assisted method was investigated and observed that the highest extract yield with distilled water was 07.84% followed by methanol (04.42%), DCM (02.40%), acetone (01.78%) and DEE (0.26%).

Out of all the processes studied, the marceration process showed lowest extraction efficiency with all solvents. During marceration, the distilled water and methanol showed comparatively same yield, whereas the

other solvents like DEE, acetone and DCM were found to be least effective with 0.04% in DEE and increased almost according to polarity with 0.10% in acetone, 0.18% in DCM, 05.90% in methanol and 05.96% in distilled water. The yield results might vary with the exposure time of microwave and ultrasonication. The difference in results from earlier studies might be due to the experimental conditions, time of exposure, applied temperature, etc.

The results are in synchronization to the previous reports where increased extraction yield was found in the Soxhlet extraction method beneath the other methods. The increased extraction yield in the Soxhlet was contributed by the repeated extraction cycles using the solvent. More compounds got dissolved in each cycle, resulting in increased yield (Patil *et al.*, 2021). The next in line was the microwave-assisted extraction that yielded more due to employment of high temperature which disrupted the cells completely and resulted in phytochemical release in the extraction solvents (Chaves *et al.*, 2020). Thus, applying higher temperature also increased percentage yield of the extracts, although it might cause destruction of some important compounds (Ngamwonglumlert *et al.*, 2017). The lower extraction yield in other extraction process might be due to low applied temperature i.e. room temperature as compared to the Soxhlet and microwave-assisted extraction.

The results obtained for extraction of crude extracts using leaves of *D. regia*, polar solvents were found significantly better than non-polar solvents such as DEE, acetone and DCM. Analyzing the results of different extraction methods, it was shown that Soxhlet extraction yielded highest using distilled water i.e. 29.28% followed by methanol (22.04%). The extraction yield obtained using other solvents was 09.24, 07.30 and 06.74% for acetone, DCM and DEE, respectively (Table 2). Again, distilled water was found to be best solvent for extraction using microwave- assisted extraction. The extraction yield found using distilled water in microwave-assisted extraction was 23.90% followed by methanol, acetone, DCM and DEE i.e. 20.26, 13.84, 13.10 and 12.10%, respectively.

In ultrasound-assisted extraction, it was observed that distilled water had the highest extraction capacity yielding 15.56% and followed by methanol (08.94%), acetone (4.26%), DCM (0.26%) and minimum yield in DEE (0.12%). In case of maceration process, slight change in the order of extraction efficiency of solvents was observed with highest extract yield using methanol i.e. 10.84% followed by distilled water (04.40%). The other three non-polar solvents were found to be less effective for extraction yielding 03.08, 1.20 and 0.96% for acetone, DEE and DCM, respectively.

On analyzing the results, it was observed that methanolic extracts of *D. regia* obtained in maceration process were most potent for the urease inhibition (Table 3). Secondly, ultrasound- assisted extraction using acetone as solvent was investigated as least efficient for urease inhibition. The urease inhibition assay showed that extracts obtained using methanol as solvent and maceration process were most effective. Experimental conditions, time of exposure, applied temperature, etc. were the factors responsible for the fluctuations in urease inhibition potential of the extracts as compared to the earlier studies conducted on Fabaceae family. For urease inhibition studies, thiourea (73.58±0.02%) was used as standard inhibitor. The urease inhibition in maceration using methanol was observed to be 52.53±0.53% ensued by DCM i.e. 47.49±0.51%. Extracts of other solvents was calculated to be 43.02±0.51% for acetone, 40.22±0.17% for distilled water and

Table 2. Effect of leaf extracts of *D. regia* on per cent yield using different solvents and extraction methods

S. No.	Samples (plant part)	Solvent	Per cent yield			
			Maceration	Ultrasound-assisted extraction	Microwave-assisted extraction	Soxhlet extraction
1.	Leaf	Methanol	10.84	8.94	20.26	22.04
		Diethyl ether	1.20	0.12	12.10	6.74
		Dichloro methane	0.96	0.26	13.10	7.30
		Acetone	3.08	4.26	13.84	9.24
		Distilled water	4.40	15.56	23.90	29.28

Table 3. Effect of stem extracts of *D. regia* on urease inhibition potential (% inhibition) using different solvents and extraction methods

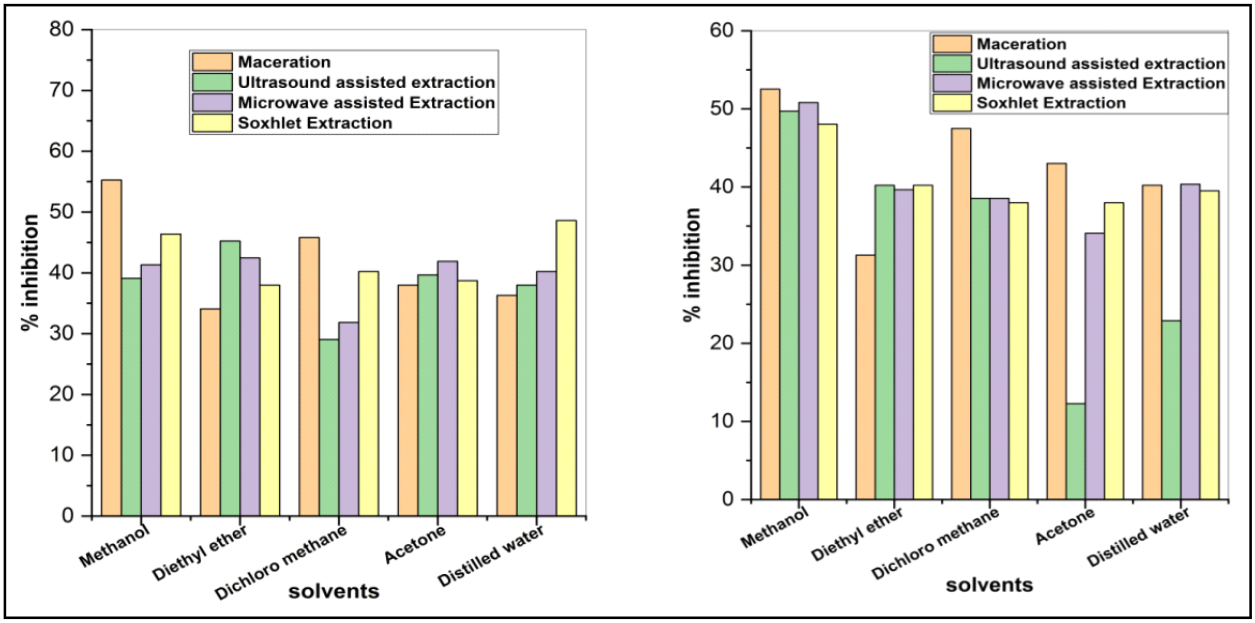
S. No.	Samples (plant part)	Solvent	Per cent yield			
			Maceration	Ultrasound assisted extraction	Microwave assisted extraction	Soxhlet extraction
1.	Stem (young)	Methanol	52.53±0.53	49.72±0.14	50.83±0.17	48.04±0.34
		Diethyl ether	31.28±0.43	40.22±0.17	39.66±0.33	40.22±0.41
		Dichloro methane	47.49±0.51	38.55±0.40	38.55±0.31	37.99±0.37
		Acetone	43.02±0.51	12.29±0.42	34.08±0.32	37.99±0.30
		Distilled water	40.22±0.17	22.90±0.20	40.37±0.19	39.51±0.30

31.28±0.43% for DEE. Using microwave-assisted extracts, the urease inhibition potential was detected to be highest for methanol i.e. 50.83±0.17% afterward distilled water (40.37±0.19%), DEE (39.66±0.33%), DCM (38.55±0.31%) and acetone (34.08±0.32%). On analyzing the urease inhibition activity of the extracts developed by ultrasound assisted extraction, methanol was observed as best solvent having 49.72±0.14% of inhibition. The extracts of DEE, DCM and distilled water showed 40.22±0.17, 38.55±0.40 and 22.90±0.20% inhibition of urease. Acetone was found to have least inhibition with 12.29±0.42% in case of ultrasound-assisted extraction. Among the extracts obtained in Soxhlet extraction, again methanolic extracts were found to be most potent for urease inhibition i.e. 48.04±0.34% followed up by DEE (40.22±0.41%), distilled water (39.51±0.30%), acetone (37.99±0.30%) and DCM (37.99±0.37%). It can be estimated from the observations that polarity did not affect much for the urease inhibition. The extracts of non-polar solvents like DEE and DCM were also having considerable inhibition following the methanolic extracts.

Urease inhibition potential of different leaf extracts of *D. regia* were investigated using different extraction processes and solvents (Table 4 and Fig. 2). According to the results, the extracts obtained by the combination of maceration process with methanol were found to have highest inhibition potential, while least inhibition was shown by DCM extracts of ultrasound-assisted extraction. In case of maceration, the urease inhibition efficiency of the methanolic extracts was 55.28±0.37% which was followed by DCM (45.81±0.40%), acetone (37.99±0.13%), distilled water (36.31±0.23%) and DEE (34.08±0.31%). DEE was found to have slightly high urease inhibition activity of 42.46±0.28% using microwave-assisted extraction. Inhibition potential of other solvent extracts was evaluated as 41.90±0.29, 41.34±0.57, 40.22±0.18 and 31.84±0.15% for acetone, methanol, distilled water and DCM, respectively. Contradictorily, DEE was found to be better solvent in ultrasound extraction with 45.25±0.34% efficiency in urease inhibition followed by acetone (39.66±0.45%), methanol (39.11± 0.23%) and distilled water (37.99±0.31%). The urease inhibition potential of DCM extracts was found to be least i.e. 29.05±0.66%. Distilled water was found to be most potent for urease inhibition in Soxhlet extraction i.e. 48.60±0.19% followed by methanol (46.37±0.16%), DCM (40.22±0.29%), acetone (38.71±0.28%) and DEE (37.99±0.49%).

Table 4. Effect of leaf extracts of *D. regia* on urease inhibition potential (% inhibition) using different solvents and extraction methods

S. No.	Samples (plant part)	Solvent	Per cent yield			
			Maceration	Ultrasound assisted extraction	Microwave assisted extraction	Soxhlet extraction
1.	Leaf	Methanol	55.28±0.37	39.11±0.23	41.34±0.57	46.37±0.16
		Diethyl ether	34.08±0.31	45.25±0.34	42.46±0.28	37.99±0.49
		Dichloro methane	45.81±0.40	29.05±0.66	31.84±0.15	40.22±0.29
		Acetone	37.99±0.13	39.66±0.45	41.90±0.29	38.71±0.28
		Distilled water	36.31±0.23	37.99±0.31	40.22±0.18	48.60±0.19



(a) Leaf extract (*D. regia*) **(b) Stem extract (*D. regia*)**

Fig. 2. Graph showing comparison of % urease inhibition using different methods and solvents.

The results were found to be in strong agreement with the previous reports showing that the alcoholic extracts prepared by maceration had significant urease inhibition potential as (Saleem *et al.,* 2019). Though the polar extracts showed considerable urease inhibition potential in comparison to other extracts yet few reports also revealed the high urease potential of acetone extracts prepared by maceration in different plant species (Prakash and Sagar, 2021). The urease inhibition efficiency of different extracts varied greatly with the plant type, extraction process, solvent used and the plant part used for the study (Zahid *et al.* 2015). Urease inhibitors involve the various categories with different functional groups (alcoholic or acidic or nitric) found to be responsible for difference in the activity of solvent extracts. The presence of dissimilar groups greatly influenced the solubility of compounds in different extracting solvents which corresponds to variation in urease inhibition efficiency of extracts obtained by different extraction methods, solvents and plant parts (Cirkovic Velickovic and Stanic-Vucinic, 2018).

CONCLUSION

The study concluded that extracts of *D. regia* were found to have significant urease inhibition activity. The results showed that the Soxhlet extraction process and polar solvents (distilled water and methanol) were best for extraction as maximum yield of 13.88 and 13.78% for stem in methanol and distilled water, respectively, and 29.28% for leaf in distilled water was achieved followed by microwave-assisted extraction yielding 11.42% for stem and 23.90% for leaf. Ultrasonication and maceration were not efficient for extraction. In urease inhibition studies, maceration process and methanol exhibited maximum urease inhibition with 55.28±0.37% for leaf and 52.53±0.53% for the stem followed by Soxhlet process with distilled water showing 48.60±0.19% for leaf extract and microwave-assisted extraction with methanol exhibiting 50.83±0.17% inhibition. The outcomes of the study indicated that out of various combinations of solvents and processes, Soxhlet method with polar solvents i.e. distilled water and methanol was found best for extraction and maceration with methanol was found best for urease inhibition. Anonymously, acetone and DCM were found to be less efficient with ultrasound-assisted extraction for urease inhibition.

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