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Phytochemical Profiling and Quantification of Premna herbacea Roxb. Extracts Using Successive Solvent Extraction.

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ABSTRACT

Premna herbacea Roxb is an unconventional accepted medicinal plant with a long-standing therapeutic potential known to have; however, a scientific validation of the phytochemical composition is limited. The aim of the investigation carried out was to determine the effect of polarity of solvents on phytochemical extraction, as well as, determine the best solvent system to be used to extract maximum bioactive compounds. Solvents with petroleum ether, chloroform, ethyl acetate, and ethanol were consecutively used to extract solvents. Qualitative phytochemical screening indicated a notable solvent dependent differences in the phytochemical profiles with non-polar extracts having fewer constituents compared to polar extracts which have increased diversity. Quantitative analysis proved that the ethanolic extract had the most total phenolic content (112.783.54mg GAE/g extract) and total flavonoid content (86.430.67mg QE/g extract), followed by the ethyl acetate, chloroform and petroleum ether extracts. It was evident that the solvent polarity and the phytochemical yield are correlated with each other indicating that polar solvents are superior in extracting antioxidant-rich compounds. The ethanolic extract was also found to have a better aptitude to nanoformulation development with phytochemical abundance and compatibility with formulation. The research provides the scientific basis of ethanol as the most efficient extraction agent and also it provides a strong phytochemical basis on the pharmaceutical use of P. herbacea especially in antioxidant and formulation related research..

Key Words: Premna herbacea, Phytochemical profiling, Total phenolic content, Total flavonoid content, Successive solvent extraction.

INTRODUCTION

The medicinal plants are a key pillar of traditional and modern pharmacotherapy as a major source of bio-active compounds to be developed into drugs, as functional foods, and nutraceutical preparations. More than eighty percent of the world population directly or indirectly depends on plant-based medicines to provide primary healthcare particularly in the developing world where plant-based products continue to be the first-line therapy against infection, inflammation, metabolic disorders, and malignancies [1]. Over the past decades, there has been a growing scientific focus on the investigation of herbal medicine in line with growing apprehensions about the safety, cost effectiveness, and resistance of synthetic medicines in the long-term. The plant secondary metabolites (phenolics, flavonoids, alkaloids, terpenoids, tannins, and glycosides) have various pharmacological effects, such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, neuroprotective, and anticancer properties [2-4]. Of them, the phenolics and flavonoids have received a closer interest because of their strong free- radical scavenging ability and multifaceted therapeutic effectiveness [5].

The chemical scaffolds of almost 50 percent of all the clinically approved drugs, including anticancer agents and antibiotics, have always come from natural products [6]. The complexity of phytochemical molecular structures makes them difficult to synthesize by strictly synthetic chemistry, which makes plants a highly special source of lead-compounds. In addition, there is also a synergistic effect of various phytoconstituents found in crude extract that generates greater pharmacological activity compared to isolated compounds, which underscores the significance of whole-plant studies [7]. However, to move the plant-based therapeutics beyond traditional use to evidence-based medicine, they should be systematically scientifically validated by phytochemical characterizing and quantifying them..

The first and the most vital step of phytochemical exploration is extraction because the nature and amount of extracted phytoconstituents directly rely upon the extraction methodology used. Polarity, molecular size, hydrogen - bonding capacity, and structural diversity control the solubility of bioactive compounds [8]. Solvents system performance, therefore, is a key to the solvent extract composition and biological potential. A solvent usually is not enough as a solvent to recover all the phytochemicals since the range of the polarity of constituents of plants is broad [9]. Hence, reiterated solvent extraction, which positions solvents progressively in descending order of polarity, provides a more reasonable and all inclusive method of phytochemical isolation [10]. This technique allows the sequential removal of non-polar lipids and terpenoids, then moderately polar flavonoids and phenolics and finally the highly polar glycosides and tannins.

Initially, successive Soxhlet extraction has been a centralized approach of any phytochemical study due to its reproducibility and scalability, and effectiveness in exhaustive extraction [11]. The frequently used solvents include petroleum ether, chloroform, ethyl acetate, and ethanol, and they have a broad polarity window, which makes them the perfect choice as a step-wise phytochemical solvent [12]. Ethanol, especially has come out as a pharmaceutically relevant solvent because of its low toxicity, environmental friendliness, and high polyphenolic extractions [13]. A number of studies indicate that ethanolic extracts often have high antioxidant, antimicrobial, and cytotoxic properties, which is mainly because of the greater phenolic and flavonoid content [14,15]. Although these documented benefits are recorded, the comparative solvent-efficiency studies are still limited in relation to a great number of medicinal plants.

Premna herbacea Roxb. is a comparatively unexplored medicinal plant (family Lamiaceae or formerly known as Verbenaceae), traditionally used in several parts of India in the treatment of fever, inflammation, digestive disorders and hepatic diseases [16]. Ethnomedical records show that it was used as a febrifuge, analgesic and anti-rheumatic in ancient recipes [17]. Early pharmacological studies have described antioxidant and antimicrobial activity as well as hepatoprotective activities of different species of the genus Premna [18,19]. However, the scientific sources on the overall phytochemical profiling and quantitative analysis of P. herbacea are limited especially in the context of solvent-specific extraction behaviour.

By screening phytochemicals, it is easy to identify broad groups of secondary metabolites in medicinal plants and has a background framework of information on therapeutic potential [20]. Nevertheless, the development of pharmaceuticals requires more than just qualitative detection since the biological activity usually has an inverse dependence on the concentration. The quantitative determination of the total phenolic and flavonoid content becomes nowadays one of the common indicators of the antioxidant potential and is used to predict bioactivity [21]. Phenolic compounds also act as hydrogen donors, metal chelators, as well as redox modulators thus alleviating oxidative stress and inhibiting cellular damage [22]. Flavonoids, on the contrary, regulate the pathways of inflammation, oncogenic enzymes and apoptosis associated signalling pathways [23].

Oxidative stress is the primary factor in the pathogenesis of chronic diseases, such as cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases [24]. As a result, the plant-based antioxidants have emerged as an alternative to synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), which are suspected of causing carcinogens [25]. This has triggered an international call to research to identify natural antioxidants that have improved biosafety profile and pharmacological activity [26]. In this regard the establishing of the phenolic and flavonoid content of medicinal plants is not only an academic but also an industrial interest.

Whereas other literature on other species of Premna indicates that this group of plants has bioactive phytoconstituents, no intensive comparative study has been performed on the effects of solvent polarity on phytochemical yield of P. herbacea. Moreover, most of the current phytochemical studies use single-solvent extraction procedures, which give partial representation of the diversity of plant metabolites [27]. Systematic extraction strategies are desperately required because they allow the accurate recognition of the most suitable solvent systems to be used in the subsequent pharmaceutical development.

This current research was thus aimed at offering a detailed phytochemical analysis of P. herbacea by consecutive solvent extraction methods. The main aim was to examine the effect of the polarity of the solvent on the qualitative distribution of phytochemicals and quantitative recovery of phenolics and flavonoids in petroleum ether, chloroform, ethyl acetate and ethanol extracts. This work will attempt to develop a powerful phytochemical fingerprint of the plant by combining qualitative screening and quantitative estimation. It will also help in formulation and pharmacological research on this species in the future by the identification of the most appropriate solvent system to obtain the optimal bioactive.

It is hoped that this research will add value to the scientific understanding of P. herbacea, (i) because it will increase the scientific knowledge on this species, (ii) because it will illustrate the impact of the solvent polarity on recovering phytochemicals and (iii) because it will provide validated phenolic and flavonoid data to evaluate drugs. It is expected that the findings will be used as a reference guideline in choosing the best extraction method of the medicinal plants and to trigger the next line of pharmacological and formulation-oriented studies on this promising herbal candidate.

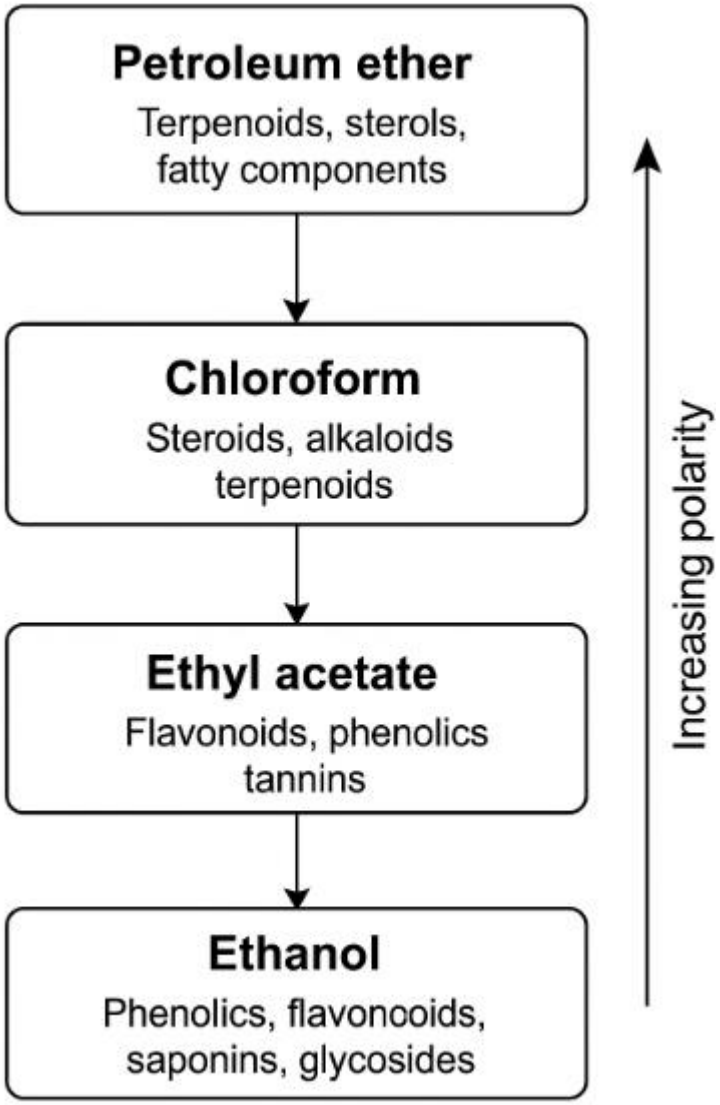

Figure 1 Effect of Solvent Polarity on Phytochemical Recovery from Premna herbacea Roxb.

2. MATERIALS AND METHOD

2.1 Collection and Authentication of Plant Material

The vegetation material is collected and authenticated using the 7-step method of herbal medicine.Collection and Authentication of Plant Material Collection and Authentication of Plant Material: The vegetation material is gathered and identified by the 7-step method of herbal medicine.

The aerial fresh and healthy parts of the herb Premna herbacea Roxb. were collected at the optimal growing season in its natural habitat to guarantee high levels of phytochemicals. The choice of the material was carried out on the basis of strict morphological rules and exclusion of any visible signs of the disease or mechanical wear. To prevent the presence of moisture and microbial contamination, the extraneous substances such as soil particles and alien detritus were carefully removed by hand immediately after the collection and samples were transported to the laboratory in sterile, ventilated receptacles.

A qualified taxonomist was used to carry out botanical authentication using standard botanical keys and floristic references. To allow eventual confirmation and assure the reproducibility of the investigation, a specimen voucher with the collection details marked on it was deposited in an institutional herbarium.

2.2 Chemicals and Reagents

The reagents and solvents as well as auxiliary chemicals obtained in this study were of the analytical grade and obtained through certified commercial sources. Sequential extraction solvent system included petroleum ether, chloroform, ethyl

acetate and ethanol that were selected based on their polarity range so that phytoconstituents across a wide range of chemical classes are recovered.

The qualitative phytochemical assays were performed on the use of the Dragendorff, Mayer, and Wagner reagents, ferric chloride, lead acetate, gelatin, aluminium chloride, acetic anhydride, concentrated sulfuric and hydrochloric acid and sodium hydroxide to identify the major phytochemical classes, namely alkaloids, flavonoids, phenolics, tannin, terpenoids, saponins, glycosides, and steroids as per the standard phytochemical

FolinCiocalteu reagent and sodium carbonate were used to measure the total phenolic content at a quantitative level, and the reagents used to measure the total flavonoid content were the aluminium chloride, sodium nitrite and sodium hydroxide. Gallingic acid and quercetin were taken as reference standards to build up calibration curves. The working solutions were all freshly made using distilled or deionized water and glassware was carefully washed and calibrated before use to provide precision in experiments.

2.3 Procedure of Drying, Powdering and storage.

Aerial parts of the newly harvested plant of Premna herbacea Roxb. were washed in distilled water to remove the soil and impurities attached to the aerial parts of the plant. The ensuing shade drying was done at ambient temperature and in a well-ventilated environment to prevent the exposure to direct sunlight which has been known to initiate photodegradation of thermolabile and photosensitive substances like phenolics and flavonoids [28]. Shade drying also reduces the enzyme oxidation and preserves bioactive secondary metabolites than sun drying or oven drying.

The process of drying continued until no change in weight was recorded which is a sign that all moisture was removed. This dry substance was then mechanically grinded in a laboratory level grinder to bring about a rough, homogeneous powder. Powdering increased the surface area thus increase solvent penetration and efficacy in mass transfer during the extraction process [29]. The product is then sieved to achieve the desired homogeneity of the particle size distribution, a crucial value to be used to increase extraction repeatability.

The powdery substance was placed in the non-reactive glass containers and kept in a cool, dry and dark place to protect the product against light, humidity, and oxygen. These conditions were preserved to prevent the growth of microbes, oxidation, and chemical breakage of the phytochemical constituents before extracting them [30].

2.4 Successive Solvent Extraction (Soxhlet Method)

The fractionation of bioactive compounds of *Premna herbacea* was performed in a Soxhlet apparatus, using solvents of increasingly different polarity, petroleum ether, chloroform, ethyl acetate, and ethanol to isolate metabolites of decreasing non-polarity (non-polar lipids), medium polarity (phenolics and glycosides) [31]. The method produces a total chemical profile and improves phytochemical recovery as compared to single-solvent extraction.

Powdered plant material was weighed and put into a cellulose thimble and inserted into the main chamber of the Soxhlet extractor. The extraction cycles lasted 6 -8 h each or till the siphoned solvent was colorless, which indicated exhaustive extraction [32]. The same amount of time taken during extraction guaranteed the same recovery of target compounds within solvent fractions.

The solvent extracts were filtered and concentrated on a rotary evaporator at a pressure lower than 45 0 C to avoid thermal degradation of labile phytochemicals and facilitate solvent extraction in the solvent extracts [33]. Further extraction was done to obtain concentrated extracts which were weighed to get extractive yield.

The dried extracts were kept in airtight, amber containers at 4 o C prior to further analysis. Low temperature storage reduced the effects of chemical degradation and retained phytoconstruential stability of phytoconstituents to be subsequently tested qualitatively and quantitatively [34].

Phytochemical screening was performed on pre-existing compounds present in the extracts, enabling the identification of phytochemical active components in the extracts.

2.5 Preliminary Phytochemical Screening

Phytochemical screening was conducted on the pre-existing compounds existing in the extracts where phytochemical active compounds were identified in the extracts.

To determine the presence of the major bioactive secondary metabolites, preliminary qualitative screening of the successive solvent extracts of Premna herbacea Roxb. was done. Standard qualitative pharmacognostic techniques were used, whereby, visual chromogenic changes or precipitates formation were used as a marker of positive reactions [35]. Screening was done against alkaloids, flavonoids, phenolics, terpenoids, tannins, saponins, glycosides, and steroids.

Detection of Alkaloids

Alkaloid identification was done with the help of Dragendorff, Mayer and Wagner reagents. The formation of an orange-brown or cream precipitate was taken as a positive outcome. These reagents get insoluble complexes with nitrogenous bases thus proving the presence of alkaloids in plant extracts [36].

Detection of Flavonoids

The identification of flavonoids was done using Shinoda test and alkaline reagent test. The presence of flavonoids was indicated by the appearance of orange, pink or red coloration after the addition of magnesium and hydrochloric acid but the appearance of yellow coloration and decolorization after acidification in the alkaline test further proved the presence of flavonoids. This response is based on the decrease of flavonoids to their coloured anthocyanidin derivatives [37].

Phenolic Compounds Detection Phenolic compounds are identified by detecting their interaction with red Agar plates containing the enzyme phenoxymethanol sulfoxide (PMS) and bovine heart cyanide (BHC), based on their antimycinolytic activity.

Phenolic compounds and tannins were revealed on the basis of the ferric chloride test, according to which the emergence of blue, green, or black color is an indication of the presence of phenolic hydroxyl group. Lead acetate was also used; the reaction of this subsequent with polyphenolic compounds gives insoluble complexes [38].

Detection of Terpenoids

The detection of terpenoids and triterpenes was done using the Salkowski and LiebermannBurchard assays. The appearance of reddish-brown or green color at the interface upon the addition of concentrated sulphuric acid was indicative of the existence of isoprenoid compounds, which can be explained by the sulfonation and further rearrangement reactions [39].

Detection of Tannins

Ferric chloride and gelatin precipitation tests were done to confirm the presence of tannin. During the assay of ferric chloride, tannins precipitate proteins and develop coloured ironphenol complexes thus allowing differentiation between hydrolysable and condensed tannins [40].

Detection of Saponins

The presence of saponin was determined through a froth and emulsification test. Constant frothing, which is caused by their surfactant activity caused by glycosidic bond between a sugar residue and an aglycone moiety was believed to be a characteristic of saponins [41].

Detection of Glycosides

The presence of general glycosides was detected after hydrolysis and then the tests of Keller-Killiani and Borntrager were used. The cardiac glycosids formed brown or blue-green rings, and anthraquinone derivatives formed pink or violet color in alkaline medium [42].

Detection of Steroids

Liebermann-Burchard and Salkowski tests were used to detect steroids and sterols. When sulfuric acid was used, the development of blue-green or red coloration proved the presence of sterol as a result of a change of structure occurring in the nucleus of cyclopentanoperhydrophenanthrene [43].

2.6 Quantitative Phytochemical Estimation

The quantitative analyses were done to determine the contents of total phenolic as well as total flavonoid compounds in solvent extract of the herb *Premna herbacea. Three measurements were performed and the figures attained were presented in mean and standard deviation. Reference compounds were used to obtain the standard calibration curves and absorbance values were obtained through UV-visible spectrophotometry [44].

2.6.1 Determination of Total Phenolic Content (TPC)

The TPC was determined according to the following procedure. A total of 0.1 mL of solution was added to 0.1g of the crude extract and mixed thoroughly by swirling both components thoroughly in the flask. 0.1 g of the crude extract was swirled thoroughly with 0.1 mL of the solution in the flask.

The FolinCiocalteu colorimetric assay was used to determine the total phenolics content (TPC). Simply, aliquots of each extract were mixed with an diluted FolinCiocalteu reagent and incubated (5 minutes) after which the addition of sodium carbonate was done to initiate reduction of phosphomolybdotungstate complexes. The absorbance at subsequent absorbance of 765nm was measured using a UV-vis spectrophotometer [45].

Gallic acid was used as a calibration standard, and a linear calibration curve was prepared between a concentration of 10 to 100 00 mg mL 1. The regression equation of this calibration curve was used to get the concentration of phenolic in the extracts. The results reported were in terms of milligrams of gallic acid equivalents per gram of dried extract (mg :: $^{-1}$ GAE) [46].

The principle of the assay is based on the electron transfer of phenolic substrates to phosphomolybdic and phosphotungstic acid complexes in an alkaline environment, which forms a blue chromophore, the absorbance of which is directly proportional to the concentration of phenolics [47].

2.6.2 Determination of Total Flavonoid Content (TFC)

The amount of the total flavonoid content (TFC) of the sequential solvent extracts of the herb Premna herbacea Roxb. was determined using aluminium chloride colorimetric assay which was an established quantitative method based on the

complexation of flavonoid with aluminium ions [48]. This assay involves the formation of acid-stable complexes of flavonoid with aluminium chloride which results in the development of yellow coloration with a direct proportional rate to the concentration of flavonoids.

In summary, an aliquot of both extracts was combined with sodium nitrite solution after which aluminium chloride reagent was added. Sodium hydroxide was also introduced after a given incubation period in order to stop the reaction and stabilize the chromogenic complex. The last volume was made up using distilled water and the spectrophotometric absorbance was used to measure absorbance at 510 nm with a suitable reagent blank [49].

The calibration standard used was quercetin. An absorbance/quercetin concentration calibration curve was plotted between absorbance and quercetin concentration, 10 to 100 mg mL -1. The amount of flavonoid in the extracts was determined by the regression equation based on this curve, as milligrams of quercetin equivalent per gram of dried extract (mg QE/g -1) [50].

The aluminium chloride technique is based on the development of flavonoid-aluminium complex at the keto and hydroxyl sites of flavonoid frameworks. The sensitivity and reproducibility of reaction is high to the quantification of flavonoid in a wide variety of plant matrices [51].

2.7 Statistical Analysis

All experimental determinations were performed in triplicate (n =3) unless otherwise stated. Mean and standard deviation (SD) were used to express data. The analysis of quantitative data obtained as a result of the phytochemical assays was done to ensure reliability and reproducibility.

One-way analysis of variance (ANOVA) was done in order to statistically compare various solvent extracts. In case of the presence of significant differences, proper post hoc multiple tests were used. The probability values that are below 0.05 were taken to be statistically significant [52].

Graphical analysis, regression analysis and calculation of IC 50 were done by use of GraphPad Prism software and statistical testing was done by use of IBM SPSS Statistics. These software tools facilitated correct data management, curve fitting and reliability testing as per accepted rules of conducting biomedical research [53].

3. RESULTS AND DISCUSSION

Table 3.1 Preliminary Phytochemical Screening of *Premna herbacea* Roxb. Extracts

Phytochemical	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol
Alkaloids	–	–	+	+
Flavonoids	–	+	++	++
Phenolics	–	+	++	++
Terpenoids	+	++	++	++
Tannins	–	+	++	++
Saponins	–	–	+	++
Glycosides	–	–	+	+
Steroids	+	+	–	– / +
Coumarins	–	–	+	+

(– = absent, + = present, ++ = strongly present)

The results clearly show that solvent polarity is the determining factor of extraction efficiency of phytochemicals. Non-polar compounds that include terpenoids and steroids are more readily recovered with petroleum ether, but moderately polar flavonoids and phenols can be recovered with chloroform at comparatively low intensity. Ethyl acetate exhibits strong enrichment of mid-polar phytochemicals such as flavonoids, tannin and phenols. Ethanol is the richest extract with nearly all groups of phytochemicals, such as alkaloids, saponins, and glycosides. The results affirm that ethanol is the best choice of solvent to use to achieve total phytochemical extraction in Premna herbacea.

Table 3.2 Total Phenolic and Flavonoid Content of *Premna herbacea* Extracts

Extract Type	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Petroleum Ether	**12.45 ± 0.82**	**5.34 ± 0.41**
Chloroform	**28.67 ± 1.15**	**14.56 ± 0.92**
Ethyl Acetate	**64.22 ± 2.31**	**38.94 ± 1.44**
Ethanol	**112.78 ± 3.54**	**86.43 ± 2.67**

There was an important effect of polarity on the content of phenolic and flavonoids. The ethanolic extract contained the highest total phenolic content (TPC) and total flavonoid content (TFC), which meant that it had the greatest amount of antioxidant polyphenols. Ethyl acetate showed moderate recovery with petroleum ether showing low levels. Since both phenolics and flavonoids can play a significant role in antioxidant, anti-inflammatory and anti-cancer activities, these results form a solid scientific basis of considering the ethanol extract as the priority in future biological studies.

Table 3.3 Basis for Selection of Extract for Nanoformulation

Parameter	Observation
Best solvent	**Ethanol**
Highest TPC & TFC	**Yes**
Rich qualitative profile	**Yes**
Presence of bioactives	**Alkaloids, flavonoids, tannins, saponins**
Suitability for formulation	**Excellent**
Intended application	**Anticancer studies**

Ethanol was selected to nanoformulate as the best solvent due to its ability to extract phytochemicals better, and broad phytochemical coverage. Its ability to extract high levels of therapeutically significant metabolites makes it most ideal in the formulation development and antitumor testing.

3.3 Total Flavonoid Content (TFC) Analysis

Table 3.3 Total Flavonoid Content of *Premna herbacea* Roxb. Extracts

Extract Type	Total Flavonoid Content (mg QE/g extract)
Petroleum Ether	**5.34 ± 0.41**
Chloroform	**14.56 ± 0.92**
Ethyl Acetate	**38.94 ± 1.44**
Ethanol	**86.43 ± 2.67**

The ethanolic extract had a high level of flavonoid, hence revealing a higher extraction of polar flavonoid sub-elements. Ethyl acetate gave a moderate level of recovery, but the chloroform and petroleum ether gave significantly low recoveries. These findings support the fact that flavonoids are more soluble in polar media. Figure 1 shows a sharp increasing trend of petroleum ether to ethanol, which has been visually supported to demonstrate a polarity-dependent trend in flavonoid extract. Ethanol has the best hydrogen-bonding ability that enhances the ability of hydroxyl-rich flavonoids to be solubilized, which forms the basis of its success in phytochemical extraction.

3.4 Correlation Between Solvent Polarity and Phytochemical Yield

Table 3.4 Relationship Between Solvent Polarity and Phytochemical Distribution

Solvent	Relative Polarity	Qualitative Richness	TPC Level	TFC Level
Petroleum ether	**Low**	**Poor**	**Very low**	**Very low**

Chloroform	**Moderate**	**Moderate**	**Low**	**Low**
Ethyl acetate	**Mid-polar**	**High**	**Moderate**	**Moderate**
Ethanol	**High**	**Very high**	**Highest**	**Highest**

Close relations between the yield of phytochemicals and solvent polarity were observed. Non-polar petroleum ether extraction did not produce any phenolic or flavonoid compounds and only extract lipophilic constituents, hence resulted in insignificant yields. Chloroform, a substance with moderate polarity gave moderate recovery. However, ethyl acetate and ethanol produced the best amounts of phytochemicals, which supports the claim that polar solvents work better in extracting phytochemicals with phenolics and flavonoid compounds. In practice, ethanol is suggested to be used in phytopharmaceutical studies due to its high efficiency and safety and its ability to be used in formulations. The data can provide useful guidelines to the solvent choice in natural product research and drug design.

3.5 Scientific Justification for Ethanol as Ideal Solvent

Table 3.5 Justification for Selecting Ethanol as Optimal Extraction Solvent

Parameter	Observation from Study	Scientific Implication
Phytochemical richness	**Ethanol extract showed strongest presence of flavonoids, phenolics, tannins, saponins, glycosides, and alkaloids**	**Indicates broad-spectrum phytochemical extraction**
Total Phenolic Content	**Highest TPC (112.78 ± 3.54 mg GAE/g)**	**Confirms superior recovery of antioxidant compounds**
Total Flavonoid Content	**Highest TFC (86.43 ± 2.67 mg QE/g)**	**Indicates maximum flavonoid solubilization**
Suitability for nanoformulation	**Ethanolic extract selected for PLGA NPs, phytosomes, and lipid nanoparticles**	**Demonstrates formulation compatibility**
Stability	**Extract stable under 4°C storage**	**Suitable for long-term handling**
Downstream application	**Used for anticancer formulation development**	**Indicates pharmaceutical relevance**

Ethanol was determined as the most effective extraction solvent, which can be explained by the fact that it was able to dissolve a wide range of phytochemicals. The ethanolic extract had the highest qualitative richness, and highest quantitative recovery of total phenolics and flavonoids compared to petroleum ether, chloroform and ethyl acetate. These results indicate that ethanol has a strong molecular affinity to polar phytoconstituents, as this is due to its ability to form hydrogen-bonds and also because of its amphiphilic nature, thus enabling it to extract moderately polar and highly polar phytoconstituents.

Regarding safety, it is generally accepted that ethanol is a benign solvent because of its low toxicity, biodegradability, and approvals of the use as a pharmaceutical solvent by governments. Ethanol has few health and environmental risks, unlike chloroform and petroleum ether, which makes it safe to use on large-scale phytochemical manipulation.

When it comes to formulation compatibility, ethanol has proved to be a universal and excellent solvent in all nano-delivery systems that were designed during this study including polymeric nanoparticles, phytosomes, and lipid-based formulations. The introduction of the ethanolic extract into various delivery systems confirms that it is chemically stable, compatible as a formulation, and has therapeutic value.

4. CONCLUSION

The current study has revealed that Premna herbacea Roxb. is a rich phytochemical medical plant, with high levels of qualitative and quantitative diversity of biological materials. Repeat solvent extraction showed that phytochemicals distributed in a clear polarity-dependent manner with limited components being extracted by non-polar solvents and more complex phytochemical profiles being obtained by polar solvents. Ethanol was found to have the best extraction efficiency since it was the only solvent which extracted the maximum amount of phenolics and flavonoids. The ethanolic extract presented the widest range of secondary metabolites which included alkaloids, tannins, saponins, and glycosides. The use of ethanol as the most suitable solvent to phytoconstituents with high antioxidant properties was confirmed by quantitative analysis. These results form a strong phytochemical platform to proceed with pharmaceutical research on this plant. The

research has scientific justification of solvent choice in herbal drugs formulation. The ethanolic extract is highly suggested in the future formulation and biological research such as nanopharmaceutical and anticancer research based on its ability to extract and its bioactive composition.

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